המועצה המדעית לישראל

BULLETIN

OF THE

RESEARCH COUNCIL

OF ISRAEL

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

NOTICE TO CONTRIBUTORS

Contributors to the *Bulletin of the Research Council of Israel* should conform to the following recommendations of the editors of this journal in preparing manuscripts for the press.

Contributions must be original and should not have been published previously. When a paper has been accepted for publication, the author(s) may not publish it elsewhere unless permission is received from the Editor of this journal.

Papers may be submitted in the following languages: English, French and Russian.

MANUSCRIPT

General

Papers should be written as concisely as possible. MSS should be typewritten, on one side only, and double-spaced with side margins not less than 2.5 cm wide. Pages, including those containing illustrations should be numbered.

The Editor reserves the right to return a MS to the author for retyping or any alterations. Authors should retain copies of their MS.

Spelling

Spelling should be based on the Oxford Dictionary and should be consistent throughout the paper. Particularly geographic and proper names should be checked for approved forms of spelling or transliteration.

Indications

Italics

All symbols and text to be italicized should be underlined.

Capitals

Capital letters should be capitalized in the MS.

Stopping

Words to be stopped should be s p a c e d out in the MS.

Other specifications

Any other variations in type size or character should be indicated clearly in a legend preceding the MS.

Mathematical script

Special care should be taken to record clearly relative height of symbols to the line. This is often more easily achieved in legible handwriting rather than typing. Indices and subscripts should be accurately placed. As far as possible formulae should be confined to one line, e.g. $\frac{1}{x}$ should rather be written $1/x$.

Greek letters should be indicated in a legend preceding the MS as well as by a pencil note in the margin, upon first appearance in the text. When there is any room for confusion of symbols, they should be carefully differentiated, e.g. the letter "l" and the figure "1"; "O" and "0".

Mathematical Punctuation

Decimal division is indicated by use of a full stop on the line, e.g., 1.000 (one, accurate to the third place). Division of thousands is made by use of a comma, e.g., 1,000 (one thousand). Multiplication is indicated by a full stop centrally placed, e.g. $8 \cdot 10^{12}$.

Abbreviations

Titles of journals should be abbreviated according to the *World List of Scientific Periodicals*.

Units are used in the abbreviated form, in the singular, and are not followed by a full stop (only in. is followed by a full stop). The following is a list of the more common symbols: mm cm m km cm^2 m^2 μg mg g kg sec min hr °K °C

Summary

Every paper must be accompanied by a brief but comprehensive summary. Although the length of the summary is left to the discretion of the author, 3% of the total length of the paper is suggested.

BULLETIN

OF THE

RESEARCH COUNCIL OF ISRAEL

CONTENTS

A COMPARATIVE STUDY OF CERTAIN *CLOSTRIDIA* FROM FLAX-RETTING LIQUORS IN ISRAEL

ESTHER HELLINGER

Daniel Sieff Research Institute, Weizmann Institute of Science, Rehovot

SUMMARY: Four distinct spore-forming organisms were isolated from local flax-retting liquors, an orange pigmented clostridial anaerobe, a plectridial anaerobe, a facultative anaerobe and a non-pigmented clostridial anaerobe. Pure cultures of these organisms were fully compared, morphologically and physiologically, with certain known bacilli from other retting liquors and their nomenclature and taxonomy discussed.

The plectridial anaerobe is described as a new variety of *Clostridium pectinovorum* Stoermer and named *Cl. pectinovorum* variety *pseudoplectriforme*.

The pectolytic and also the flax-retting abilities of these and certain aerobic organisms were tested in experiments with purified lemon pectin, and with sterilised flax straws. It was shown that the orange pigmented form, identified as *Cl. aurantibutyricum* was a highly efficient retting organism comparable to *Cl. felsineum* and was superior to all other types of pectin splitting bacteria.

INTRODUCTION

Direct microscopic examination of the bacterial flora in four natural retting liquors in Israel revealed the preponderance of various clostridial, including plectridial, forms common to all liquors (Hellinger, Avigan, Lewin, 1951). Streaks made directly from the retting liquors or from initial subplants on cereal mashes onto several successive test-tube slants of nutrient glucose agar, anaerobically sealed by Dorner and Ritter's method (1933) kept at 30°C, produced various types of colonies in 24—48 hours. Two types predominated, namely an orange pigmented form, and a semi-translucent somewhat opalescent colony form. Relatively few opaque colonies and an occasional well raised compact, glutinous, colony were also observed. Subsequently four distinct spore-forming organisms were isolated in pure culture and listed as follows:— Hf1— an orange pigmented anaerobe; Hf2—a plectridial anaerobe; Hf4—a facultative anaerobe; Hf5—a non-pigmented anaerobe.

To facilitate their identification and classification, these organisms were fully diagnosed and compared with known strains of the following organisms:— the orange butanol organism, *Cl. felsineum* Carbone (Carbone and Tombolato, 1917); the orange butyric acid organism, *Cl. aurantibutyricum*, Hellinger (1947), and our Pj strain of the plectridial butyric acid-producer, *Cl. pectinovorum* Stoermer (Weizmann and Hellinger, 1940).

Because of their natural association with flax and other vegetable fibre-plants, their role as flax-retting agents has also been studied.

IDENTIFICATION AND CLASSIFICATION

Experimental

Morphology and cultural features.   Morphological and cultural features of the organisms were studied mainly on 1% tryptone plus 0.2% glucose. The diagnostic features are recorded in Tables I and II.

Carbon metabolism.   The ability to utilize various carbon sources was determined by measuring the amounts of gas and volatile acid produced from 25 ml amounts of basal medium (1% tryptone, 0.025% sodium thioglycollate and 0.8% dry-sterilised calcium carbonate) plus 1—2% of the substance under test, sterilised in long narrow tubes (2.3 × 1.7 cm) at 110° C for 45 min. Each tube was inoculated with 0.2 ml of an active culture on basal medium plus 0.2% glucose, and connected with a water-filled burette. Results are given in Tables III and IV.

Fermentation of glucose.   The organisms were grown on 1 litre of medium consisting of 2% glucose, 1% tryptone, 0.025% sodium thioglycollate plus some small pieces of filter paper, and sterilized as above. Analyses of the fermentation products of glucose are given in Table V.

Residual sugar was determined according to Lehman—Maquenne—Schoorl (van der Haar, 1920).
Neutral solvents were distilled off from 200 ml of neutralized fermented medium. 100 ml of distillate were collected. The *acetone* content was estimated according to Goodwin's modification (Goodwin, 1920) of Messinger's method; *isopropanol* by the method of Langlykke, Peterson and McCoy (1935) and *ethanol* and *butanol* by Johnson's (1932) method.
Acetoin was estimated on 200 ml of the fermented medium and the residual medium used for estimating 2.3 butanediol, according to Kluyver, Donker and Visser't Hooft's (1925) modification of Lemoigne's method.
Volatile acids were determined from 50 ml of fermented medium acidified with phosphoric acid and steam distilled until all volatile acids were distilled over. The acids were then determined according to Duclaux, using distillation constants calculated from our own data obtained with pure acids.

Fermentation of maize mash.   To facilitate further the identification of the orange organism Hf 1 the three orange anaerobes were grown in 1.5 L flasks containing 1 litre 5% maize mash. The analyses of the products of fermentation are given in Table VI.

Fermentation of pectin.   The organisms were grown on purified lemon pectin in a nutrient medium prepared according to Potter and McCoy (1947), except that tryptone (1%) replaced the liver extract of their medium. Gas and volatile acid production were measured and the unattacked pectin recovered as calcium pectate. The results are given in Table VII.

Results

The organisms fall into a number of natural groups, as shown by the following description:

Hf 1, *Cl. felsineum, Cl. aurantibutyricum.*   The salient features of these three chromogens are: (1) orange growth on solid and liquid media, (2) rapid gelatine liquefaction, (3) active pectin fermentation, (4) acetoin produced from carbohydrates. Hf 1 clearly resembles *Cl.aurantibutyricum* and both differ from *Cl.felsineum* in producing high yields of volatile acids and only fair or slight yields of neutral volatile products (acetone and butanol) from glucose and starch mashes, and show incomplete

diastatic action on 5% maize mashes. *Cl.felsineum* shows high yields of butanol and acetone, poor yields of acids, and complete diastatic action on maize mashes.

Hf2, Cl. pectinovorum (Pj strain). These two organisms show certain features in common: (1) plectridial form of sporangia, (2) gelatine liquefaction, (3) non-production of acetoin, (4) high yields of volatile acids. Distinct differences between them are: (1) Colonies of Hf2 are slightly raised semi-translucent, may show certain variations to a somewhat creamy coloured growth; Pj strain produces well-raised, round, compact, glutinous, waxy coloured growth, hardly distinguishable from the colony form of Hf4; (2) Pj sporulates readily within 24 to 48 hrs, only typical tennis-racket-like plectridia observed (Figure 5), which are somewhat longer and appear slenderer than Hf2. The latter sporulates extremely slowly, and sometimes produces a slight number of sporangia which are not strictly plectridial (Figure 6); (3) Pj produces slight amounts, Hf2 appreciable amounts of lactic acid; (4) Pj rapidly liquefies gelatine, Hf2 shows very slow liquefaction; (5) Pj scarcely attacks pectin, Hf2 does attack pectin, but without gas and volatile acid production.

Hf4. This organism is a facultative anaerobe; develops on carbohydrate and carbohydrate-free media. Sporangia are comparatively short with centrally located spores. Colony form is well raised, round, compact, glutinous resembling the Pj strain of *Cl. pectinovorum*. It reduces nitrates, liquefies gelatine, produces catalase, emits distictive diacetyl odour from milk and carbohydrate cultures. Fermentation products from glucose in the medium tested are: appreciable yields of ethanol, small amounts of acetone, 2.3 butanediol, isopropanol, and very small amounts of acetoin; appreciable amounts of lactic acid, but very little volatile acids (chiefly acetic, traces of butyric).

Hf5. Salient features are creamy, opaque colonies; non-liquefaction of gelatine; pectin not fermented; fair diastatic action on maize mashes; produces from glucose high yields of butyric and acetic acids, but only slight yields of acetone and butanol. Acetoin is not produced.

Utilization of carbon sources. They all, more or less, actively ferment glucose, sucrose, maltose, mannose, galactose, arabinose, lactose, laevulose, raffinose, xylose, starch, glycogen, dextrin, salicin. Hf1 and *Cl.aurantibutyricum* weakly attack raffinose, which is readily attacked by *Cl. felsineum*. Hf5 readily ferments mannitol and to a slight extent sorbitol.

Hf4 in general is weak in acid and gas production. It attacks although weakly, glycerol and mannitol and fails to attack rhamnose.

None of the organisms attack dulcitol, erythritol, cellulose, calcium lactate.

Nomenclature and Taxonomy

The results of this comparative study have shown that the organisms are distinct, yet closely related groups. Hf1 has been identified as a strain of *Cl. aurantibutyricum*. Hf2 bears a close relationship yet is not identical with our Pj strain, nor strains described in the literature for *Cl. pectinovorum* Stoermer. Hf4 is obviously a strain of the specific group of facultative sporulating organisms discovered and designated by Prazmowski (1880) as *Cl. polymyxa*, but more generally known as *B. polymyxa* Prazmowski or *Aerobacillus* polymyxa Donker. Hf5 is a strain of *Cl. butyricum* Prazmowski. In order to clarify

the identification of these organisms, their nomenclature and taxonomy is fully discussed.

Generic nomenclature. Bergey's (1948) definition for the genus *Clostridium* is: Rods frequently enlarged at sporulation producing clostridial or plectridial forms. Anaerobic and micro-aerophilic. Biochemically very active. Many species ferment carbohydrates producing various acids (frequently butyric) and gas (CO_2, H_2 and sometimes CH_4). Cells possess no catalase. This definition is generally accepted by all bacteriologists, except the French, as Weinstein, Prevot and Reynaud, who adhere to Fischer's (1895) generic terminology *Plectridium* which groups together bacilli having long, slender cells and large terminal oval spores. Variable forms are not uncommon among the clostridia, even within single strains of species, and lend support to the decision of the Society of American Bacteriologists (Winslow et al. 1917 and 1920) and also Bergey that *Plectridium* is not sufficiently distinct to warrant generic differentiation.

Cl. aurantibutyricum and Cl. felsineum. The behaviour of the W.45 strain and the new isolate Hf1 on sugar and starch mashes confirms our earlier work (Hellinger, 1947) on *Cl. aurantibutyricum*, an orange, butyric-acid-producing clostridium, and supports the justification for the separation of this new species from the butylic chromogen *Cl. felsineum* Carbone. Both chromogens have many features in common. They are morphologically identical, they cause rapid liquefaction of gelatine, actively ferment pectin and produce acetoin, yet the two species differ markedly in their behaviour on glucose media. *C. aurantibutyricum* is a 'butyric' chromogen, whereas *Cl. felsineum* is a 'butylic' chromogen. *Cl. felsineum* readily attacks inulin, *Cl. aurantibutyricum* attacks inulin weakly, if at all. The pigmentation of *aurantibutyricum* tends to be somewhat deeper orange in colour than *Cl. felsineum* which tends to be yellow-orange, but this is variable and therefore unreliable as a distinguishing feature. The orange colour may fade on cereal mashes, but colonies on nutrient-glucose-agar slants anaerobically sealed do not lose their colour on exposure to air.

Cl. pectinovorum. The new plectridial isolate Hf2 shows the general features of the specific group *pectinovorum*. These organisms liquefy gelatine, are fair fermenters of glucose yielding butyric and acetic acids and only slight amounts of neutral volatile products. Acetoin is not produced. Yet, Hf2 is recognisable by a number of more or less minor features from our Pj strain of *pectinovorum*. Pj produces distinctive well raised, compact, glutinous, waxy coloured colonies which are removed whole when picked at. This colony form has remained a distinctive feature of the organism since first isolated in 1935. Beijerinck and van Delden (1904), however, describe their *Granulobacter pectinovorum* (Synonym of *Clostridium pectinovorum*) as forming 'moire'-like colonies. Hf2 generally forms large semi-translucent, slightly raised colonies. The sporangia of Hf2 are recognisable from Pj. The latter has always produced typical, long, slender tennis-racket like capitate sporangia, similar to those depicted in the literature for *pectinovorum*; Hf2 sporangia tend to be somewhat shorter and appear stouter, and although most of the plectridia are true capitate sporangia, a number of pseudo-plectridial forms may be formed, i.e. not truly capitate. Again Pj actively liquefies gelatine, whereas Hf2 liquefies gelatine very slowly, taking from 4—7 days. The two organisms distinctly differ in their ability to attack pectin. Pj scarcely attacks pectin; Hf2 does attack pectin.

The morphological, physiological descriptions and the microphotographs and draw-ings given for Fribes' plectridial organism (Winogradsky and Fribes, 1895), Stoermer's *Plectridium pectinovorum* (1903, 1904), Beijerinck and van Delden's *Granulobacter pectinovorum* (1903, 1904) and later studies by others on similar organisms (Ruschmann and Bavendamm, 1925; Donker, 1926; Weizmann and Hellinger, 1940) are so much alike that they may be considered strains of the specific group *Cl. pectinovorum* Stoer-mer. Early investigators recorded the ability of this specific group to ferment pectin and drew attention to its importance as the flax-retting agent. This ability to ferment pectin has, however, been criticized by later workers. Our Pj strain has always shown weak or no activity on pectin in synthetic media. Hf2 on the other hand has maintained its ability to attack pectin since first isolated about two years ago.

The Pj strain produces slight amounts of lactic acid confirming our earlier work with this organism (Weizmann and Hellinger, 1940). Donker observed that 2 original strains of Beijerinck and van Delden's *Granulobacter pectinovorum* which he studied did not produce lactic acid. Hf2 produces appreciable amounts of lactic acid. Weiz-mann and Hellinger (1940) recorded the production of lactic acid as a distinctive feature of *Cl. pectinovorum* var. *parvum*, so named because of its minute translucent colony form on nutrient glucose agar. But the slender tennis-racket-like plectridia of this varietal form were scarcely distinguishable from typical *pectinovorum* strains. Thus Hf2 cannot be considered identical with the varietal form *parvum*.

Cl. tertium, a plectridial form reported by Allen (1946) from retting liquors in Great Britain reduces nitrates to nitrites and does not liquefy gelatine, and thus consequently Hf2 is not identical with *Cl. tertium*.

Summarising, Hf 2 is sufficiently similar in its major characteristics to *Cl. pectinovorum* to be included as member of this specific group, but in view of its distinguishable and recognisable features we would classify our plectridial isolate, Hf2 as a variety of *Cl. pectinovorum* and suggest the nomenclature, variety *pseudoplectriforme*.

Bergey's Manual wrongly places *Cl. pectinovorum* Stoermer as a synonym of *Cl. butyricum* Prazmowski. The mistake was originally made by Bredemann (1909) who considered Stoermer's organism identical with a number of variously named butyric acid organisms and called them all *B. amylobacter*. All these organisms without excep-tion were later transferred by Bergey to the specific group *Cl. butyricum* using Praz-mowski's original nomenclature. Sufficient evidence has been given above that the recognition by the early investigators and since of *Cl. pectinovorum* Stoermer (generic synonyms: Plectridium, Granulobacter, Bacterium) as a distinct group is fully justified. The major features of *Cl. pectinovorum* are so markedly different from *Cl. butyricum* that the organisms cannot possible be regarded as identical.

B. polymyxa. Hf4 is undoubtedly identical with Prazmowski's *Clostridium poly-myxa*. This group is distinguishable from general clostridial forms by: being able to grow in the presence of air, and also on media lacking in carbohydrates; reduces nitrates; produces catalase and 2.3 butanediol. It ferments most carbohydrates with weak acid and gas production; coagulates milk; does not ferment rhamnose and sorbitol.

The early investigators as Prazmowski, Gruber (1905), Beijerinck and van Delden placed the *polymyxa* group with other clostridial forms within the genus *Clostridium* (synonym: *Granulobacter* Beijerinck and van Delden) even though the ability of the

polymyxa group to grow in presence of air was recognised. Donker (1926) supported by Kluyver and van Niel (1936) separated the *polymyxa* group from the genus *Clostridium* and placed it in a new generic group *Aerobacillus*. Porter, McClesky and Levine (1937) describe and discuss the taxonomy of these organisms. They refer to the confusion of Donker's generic nomenclature *Aerobacillus* as the term was later used by Pribram and also Janke for quite different groups of organisms. Porter et al. are of the opinion that due to priority of Donker, his designation should be adopted if the genus *Aerobacillus* is found to be desirable. McCoy and co-workers (1939) state "the *polymyxa* group is related by general morphology and many points of general physiology to the butyric acid-producing and the butyl alcohol-producing anaerobes." Bergey (1948), however designates this facultative anaerobe as *Bacillus polymyxa* and one finds this terminology is far more in common use by bacteriologists.

Cl. butyricum Prazmowski. The salient features differentiating *Cl. butyricum* Prazmowski from other butyric acid producing clostridia are: its creamy opaque colony

TABLE I

Cultural and physiological characteristics

	Hf1	Hf2	Hf4	Hf5	Cl.fel-sineum	Cl.auranti-butyricum	Cl.pectino-vorum (Pj)
Relation to O₂	Oblig. anaer.	Micro-aerophilic	facult. anaer.	oblig. anaer.	oblig. anaer.	oblig. anaer.	oblig. anaer.
Indol	0	0	0	0	0	0	0
Nitrate reduction	0	0	+	0	0	0	0
Gelatine liquifact.	+ 24-48hrs.	+ after 4 days	+ 3 days	0	+ 24-48hrs.	+ 24-48hrs.	+ 24-48hrs.
Milk (a) coagulat. (b) peptonisation (c) odour	+ + butyric	+ slight butyric	+ + diacetyl	+ 0 butyric	+ + butylic	+ + butyric	+ + butyric
Maize mash (a) pigmentation (b) gas (c) diastatic activity (d) residual starch	orange + rapid incompl. +	0 + rapid incompl. +	0 + partial +	0 + fair +	orange + rapid incompl. 0	orange + rapid incompl. +	0 + rapid incompl. +
Standard broth/agar growth	0	—	+	0	0	0	0
Catalase	0	0	+	0	0	0	0
Anaerobic growth on nutrient glucose agar slants	orange, more or less round-ed; growth sometimes occurs from centre down into agar	large semi-translucent more or less rounded	well raised, compact, smooth glutinous	creamy, opaque	as Hf1	as Hf1	as Hf4

+ positive; 0 negative;

TABLE II

Morphological characteristics

	Hf1	Hf2	Hf4	Hf5	Cl.felsineum	Cl.auranti-butyricum (W.45)	Cl.pectino-vorum (Pj)
Rods, Gram stain	+	+	variable	+	+	+	+
Length							
Minimum	2.7	1.7	3.45	3.8	3.2	2.9	2.5
Maximum	5.6	5.5	5.5	8.92	10.25	10.48	6.2
Average	3.8	3.2	4.7	5.6	5.9	5.75	4.15
Width							
Minimum	0.56	0.32	0.73	0.73	0.4	0.48	0.48
Maximum	0.73	0.64	0.94	0.91	0.8	0.73	0.64
Average	0.65	0.46	0.85	0.83	0.58	0.59	0.56
Sporangia Form	mostly spindle shaped	plectri-dial	short spindle	mostly spindle	mostly spindle	mostly spindle	plectri-dial
Length							
Minimum	4.45	6.45	2.09	3.65	4.51	3.90	6.05
Maximum	7.75	9.65	3.91	11.12	6.20	7.25	11.35
Average	5.90	7.50	3.19	6.25	5.15	5.40	8.21
Width							
Minimum	1.11		1.09	0.73	0.89	0.56	
Maximum	2.11		1.18	1.45	1.29	1.21	
Average	1.62		1.15	1.00	1.12	0.87	
Spores Location	mostly subterm.	terminal	mostly subterm.	mostly subterm.	mostly subterm.	mostly subterm.	terminal
Length							
Minimum	2.5	1.6	1.55	1.60	1.95	1.45	1.45
Maximum	3.3	2.65	2.40	2.25	2.40	2.9	2.25
Average	2.9	2.15	1.82	2.08	2.21	2.12	1.75
Width							
Minimum	0.81	0.73	0.73	0.81	0.89	0.64	0.81
Maximum	1.13	1.77	1.21	1.21	1.29	1.53	1.85
Average	1.0	1.07	0.97	1.03	1.06	1.03	1.06

form on nutrient glucose agar; non-liquefaction of gelatine; partial diastatic activity on maize mashes; production of high yields of butyric and acetic acids, and generally small yields of neutral volatile products from starch and sugar mashes; non-fermentability of pectin; acetoin not produced. Hf5 is obviously a typical strain of the type species.

The identification of new strains should present no difficulties if, as stressed in an earlier paper (Weizmann and Hellinger, 1944), the specific group *Cl. butyricum* is accepted to cover only strains which fall strictly within the diagnostic description given in recent editions of Bergey's Manual of Determinative Bacteriology (1939 and 1946) for the type species *butyricum*. This would exclude all gelatine-liquefying strains such as *Cl. pectinovorum*. Greater use of varietal names is urged, indicating as far as possible the minor easily distinguishable physiological and morphological differences freqently occuring between these forms which otherwise fall naturally within one species. For instance, a strain which shows a very high saccharolytic ability could be referred to as variety *saccharolyticum*.

TABLE III

Gas production (ml) from various carbohydrates and related substances at 30°C

	Amt. used (g)	Hf1	Hf2	Hf4	Hf5	Cl.felsineum	Cl.auranti- butyricum (W.45)	Cl.pectino- vorum (Pj)
Glucose	0.5	199	190	27	161	180	185	94
Sucrose	0.5	199	236	34	203	186	217	119
Starch	0.5	166	185	63	41	188	195	122
Maltose	0.5	202	191	34	153	166	186	108
Mannose	0.5	157	150	20	189	196	207	111
Galactose	0.5	206	186	26	181	199	218	103
Arabinose	0.5	122	164	13	174	134	197	78
Lactose	0.5	154	169	30	179	162	125	102
Laevulose	0.5	147	131	11	218	182	174	111
Raffinose	0.5	69	120	17	176	142	24	89
Xylose	0.25	108	92	13	108	64	55	63
Rhamnose	0.25	87	0	0	9	49	52	34
Glycogen	0.25	90	86	45	17	90	70	104
Inulin	0.25	23	49	24	32	82	0	0
Dextrin	0.25	87	58	11	18	59	42	81
Salicin	0.25	91	46	34	80	50	45	79
Pectin	0.25	74	3	9	0	89	79	3
Glycerol	0.25	0	0	44	4	0	0	0
Mannitol	0.25	0	0	11	30	0	0	0
Sorbitol	0.25	0	0	0	16	0	0	0

Dulcitol, erythritol, cellulose, Ca-lactate, basal medium: no gas evolution by any of the organisms.

TABLE IV

Volatile acids (ml N/10) from carbohydrates and related substances

	Amt. used (g)	Hf1 (ml)	Hf2 (ml)	Hf4 (ml)	Hf5 (ml)	Cl.felsineum (ml)	Cl.auranti- butyricum (W.45) (ml)	Cl.pectino- vorum (Pj) (ml)
Glucose	1	50.5	40.0	8.5	45.3	37.9	62.6	26.6
Sucrose	1	50.6	29.8	7.1	56.0	32.9	67.6	29.7
Starch	1	35.1	35.4	7.4	10.0	41.4	56.6	29.6
Maltose	1	45.0	43.3	7.4	47.2	39.8	58.0	30.7
Mannose	1	41.0	29.4	7.2	53.0	44.9	62.6	27.8
Galactose	1	46.4	47.6	8.3	54.7	32.6	58.1	26.7
Arabinose	1	43.1	31.6	7.1	56.5	38.9	82.7	27.8
Lactose	1	43.6	33.0	10.5	53.3	36.1	62.7	28.3
Laevulose	1	43.1	31.7	9.5	54.5	42.9	57.4	25.4
Raffinose	1	22.3	30.0	6.2	52.8	32.3	19.1	25.3
Xylose	0.5	39.1	20.2	10.3	28.3	21.4	17.8	20.9
Rhamnose	0.5	40.3	2.0	2.2	12.8	24.0	31.2	15.3
Glycogen	0.5	45.5	23.2	10.3	9.4	24.5	22.3	24.8
Inulin	0.5	7.9	16.2	5.8	12.1	32.2	—	—
Dextrin	0.5	32.4	17.8	5.7	8.2	18.7	16.8	26.4
Salicin	0.5	29.1	12.6	5.3	24.7	18.5	15.5	29.3
Pectin	0.5	57.9	3.4	7.3	2.5	40.0	56.8	5.6
Glycerol	0.5	1.3	—	6.5	9.0	—	—	—
Mannitol	0.5	0.9	—	6.3	6.7	—	—	—
Sorbitol	0.5	2.3	—	—	6.3	—	—	—

TABLE V

Products of fermentation of 1 litre of tryptone-glucose-sodium thioglycollate medium after seven days, at 30°C

	Hf1	Hf2	Hf4	Hf5	Cl.felsi-neum	Cl.auran-tibutyri-cum(W.45)	Cl.pec-tino-vorum (Pj)
Glucose fermented %	33.3	27.1	99.0	25.5	96.9	52.1	39.1
pH value	4.1	3.9	5.4	4.2	4.3	4.2	4.2
Titratable acidity (ml 0.1 N acid/10 ml)	2.1	4.5	1.8	4.7	4.6	7.7	4.0
			Products calculated in mmol./100 mmol of glucose fermented.				
Acetone	0.0	1.1	6.0	5.5	25.7	4.5	2.1
iso Propanol	0.0	0.0	9.9	0.0	1.0	0.0	0.0
Butanol	2.3	4.7	0.0	2.5	38.5	12.0	0.0
Ethanol	17.4	0.5	69.6	1.7	13.3	8.8	1.3
Acetoin	2.0	0.0	0.4	0.0	0.6	0.2	0.0
2.3 Butanediol	0.0	0.0	5.5	0.0	0.0	0.0	0.0
Butyric acid	48.5	51.0	traces	98.7	5.9	46.3	18.0
Acetic acid	53.5	41.0	3.3	41.2	29.0	92.7	54.1
Lactic acid	0.0	37.3	23.6	0.0	23.4	4.8	3.6

TABLE VI

Products of fermentation of 1 litre 5% maize mash after 7 days at 30°C

	Hf1	Cl.felsineum	Cl.aurantibutyricum (W.45)
% starch fermented	75.5	98.7	61.6
Final pH value	3.46	3.95	4.15
Titrat. acidity (ml 0.1 N acid/10ml	8.84	4.29	6.92
		% on fermented starch	
Acetone	1.17	8.00	2.50
iso Propanol	2.46	1.51	0.25
Butanol	5.37	14.87	5.49
Ethanol	2.71	5.60	1.23
Acetoin	0.15	0.11	0.33
Total neutral solvents	11.86	30.09	9.80
Butyric acid	13.65	1.02	11.37
Acetic acid	14.89	6.95	15.58
Lactic acid	0.85	1.99	0.00

TABLE VII

Fermentation of 20 ml amounts of 1% pectin medium

Organism	Gas production (ml)	Volatile acids (ml 0.1 N)	Residual Pectin (mg)	Pectin fermented (%)
Hf1	59	23.2	0.0	100
Hf2	2	1.3	57	73
Hf4	7	2.9	132	38
Hf5	0	0.0	210	1
Cl.felsineum	72	16.0	0.0	100
Cl.aurantibutyricum (W.45)	63	22.7	0.0	100
Cl.pectinovorum (Pj)	3	2.2	208	2
Control			212	
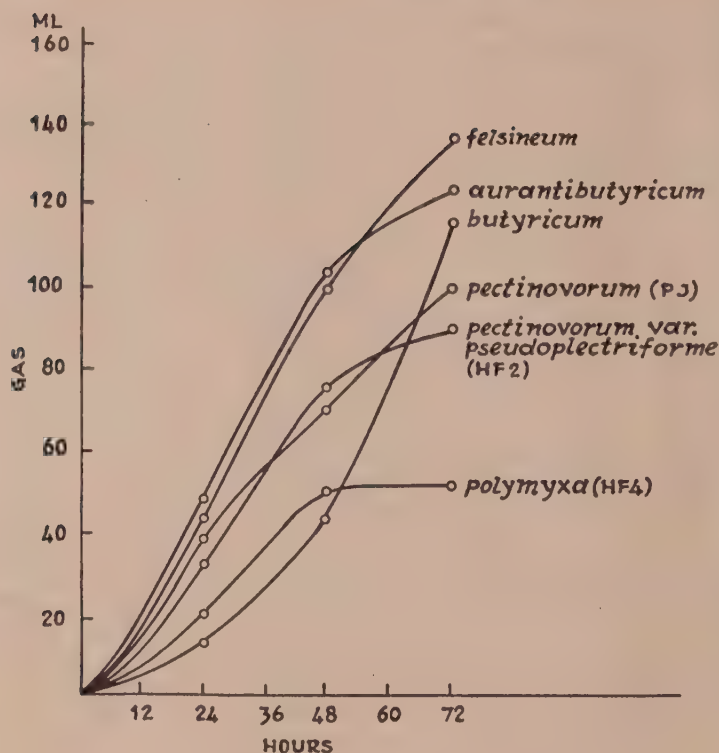
Figure 1
Gas production by various *Clostridia* from 5 g retting flax straws

FLAX-RETTING ABILITY

Certain plectridial and clostridial anaerobes have long been regarded as highly efficient biological agents responsible for the loosening and the splitting of the coarser fibre bundles within the stem, during the so-called retting process. Retting of fibre plants takes place when the stems are immersed in water, soluble carbohydrates, nitrogenous substances and other essential food substances leach out from the stems into the water, enabling the development and multiplication of the bacterial community present including those organisms which produce active pectolytic enzymes. These enzymes cause the breakdown of the pectic substances holding the cells together, thereby freeing the strong fibre bundles from the softer stem tissue. The final separation of the fibres can then be easily carried out by mechanical means.

It is of special interest to know which of the naturally occurring clostridial and plectridial organisms in local flax rets are energetic pectic-splitting organisms, and how they compare as flax retting agents with certain sporulating anaerobes which are reputed to be active retting organisms of economic importance. Consequently, the clostridial and plectridial forms isolated from local retting liquors and described in the first part of this paper were compared by simple experiments with certain anaerobic, and also aerobic organisms many of which have been cited in the literature as flax-retting organisms. Firstly, they were compared in their ability to utilise pectin in artificial media,
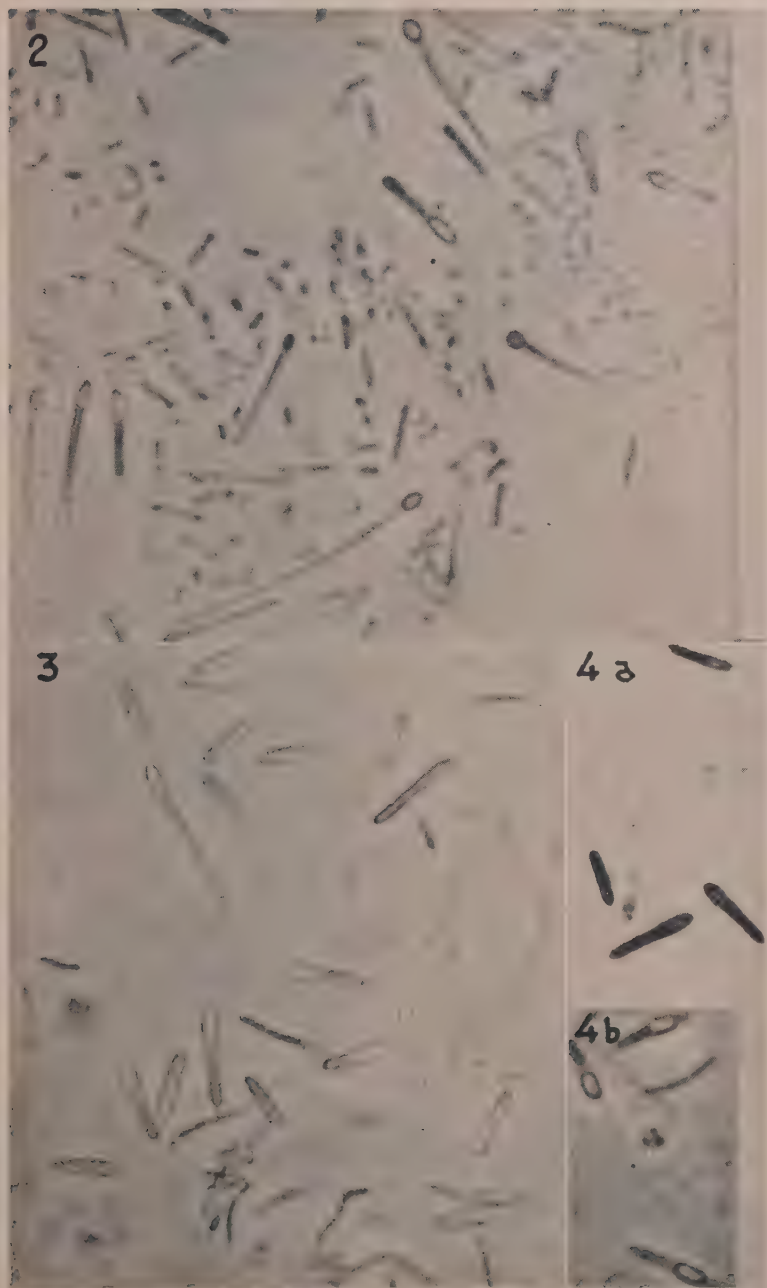
Figures. 2–4: 2. Smear of local flax-retting liquor (unstained) × 1550.   3. *C. felsineum:* 48 hrs, growth on 5% maize mash at 30°C × 1550.— 4. *Cl. aurantibutyricum* (a) 3 days growth on 5% maize mash at 30°C (Gram stain) × 1550; b) 48 hrs on yeast-glucose medium (unstained) × 1550
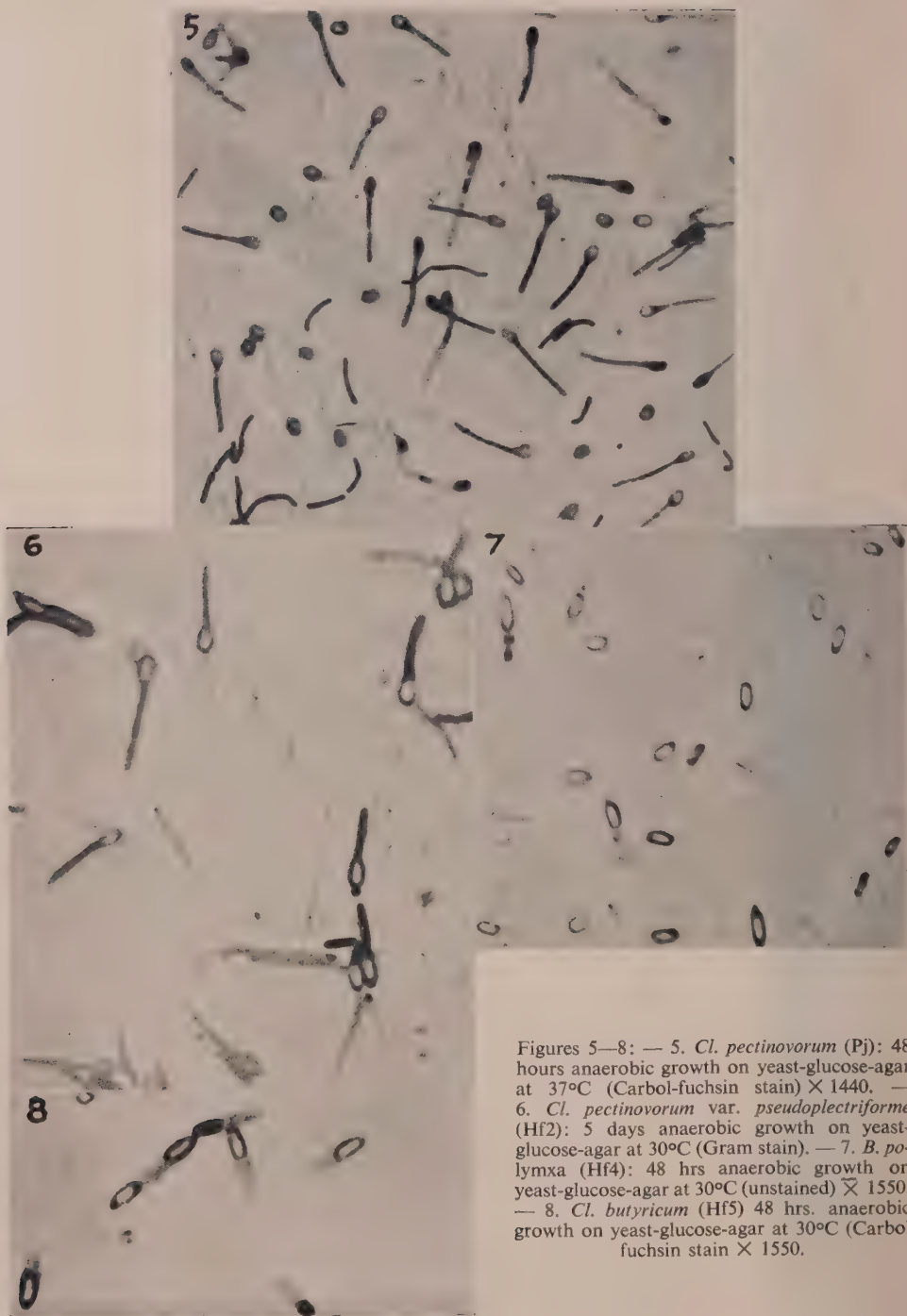
Figures 5—8: — 5. *Cl. pectinovorum* (Pj): 48 hours anaerobic growth on yeast-glucose-agar at 37°C (Carbol-fuchsin stain) × 1440. — 6. *Cl. pectinovorum* var. *pseudoplectriforme* (Hf2): 5 days anaerobic growth on yeast-glucose-agar at 30°C (Gram stain). — 7. *B. polymxa* (Hf4): 48 hrs anaerobic growth on yeast-glucose-agar at 30°C (unstained) × 1550. — 8. *Cl. butyricum* (Hf5) 48 hrs. anaerobic growth on yeast-glucose-agar at 30°C (Carbol fuchsin stain × 1550.

secondly in their ability to ret flax straws. Finally their relative merits as flax-retting agents of economic importance are discussed.

A series of experiments on the retting of whole flax straws under different experimental conditions for the production of high grade quality fibre is in progress in our laboratory, and will be reported in due course.

Experimental

Pectin. The utilisation of pectin by the following organisms was compared on 1% puri-fied lemon pectin in an artificial medium prepared as mentioned in the earlier part of this paper: *Cl. aurantibutyricum* (W.45 and Hf1 strains), *Cl.felsineum* (A.T.C.C. 7678), *Cl.pectinovorum* var. *pseudoplectriforme* (Hf2), *B.polymyxa* (Hf4), *Cl.butyricum* (Hf5), *B.megatherium* (Bc5), *B,mycoides* (Bc7), *B.undulatus* (Bc8), *B.mesentericus* (Bc9), *B. mesentericus vulgatus* (Bc10).

The anaerobes were subplanted into long tubes with 20 ml medium. The aerobes were subplanted into long tubes with 10 ml medium and placed in a sloping position to en-courage their growth and activity. The unattacked pectin was determined as calcium pectate by a slight modification of Hinton's (1939) method. The results are briefly recorded in Table VIII.

Retting of flax straw pieces.   Retting ability was tested as follows: Approximately 5 g of flax straw pieces (19 cm in length) were placed in long test-tubes (30 × 2.5 cm) with 2 ml water, plugged and steam sterilised for 1 hr on three consecutive days. In testing the anaerobic organisms, an active 24 hr old test tube culture of the organism on 8 ml tryptone (1%) glucose (0.2%) medium was poured into 85 ml of sterile tap water from which the dissolved oxygen had been expelled prior to use, and the aqueous culture poured on to the flax straws. An alcohol-cleaned cylindrical piece of solid glass was placed on the straws to keep them well immersed. The glass tube was then connected with a water filled gas burette and kept at 30° C for 72 hrs or longer. Gas production, final pH values and titratable acidities were recorded.

The aerobes were tested on the straw pieces by pouring 85 ml water plus bacterial culture onto the flax straws for about 18 hrs. Most of the aqueous culture was then poured off leaving about 20 ml, and the tube sloped and repeatedly rolled to keep the straws moist with the bacterial culture.

After 72 hrs a ret test was carried out simply by placing 4 straw pieces in a long test-tube, covering well with boiling water, and after a minute or so vigorously shaking. Well loosened fibres in the completely retted straws would on vigorous shaking be completely freed from the stem, and become so entangled as to form a mass or ball of fibres, generally about midway from the stem ends, leaving behind the bare woody core of the stem. The relative quantity of separated fibres and their entanglement indicates the degree of retting, whereas inability of the organism to ret is clearly de-monstrated by a complete non-separation of the fibres from the stems, i.e. stems remain intact.

Results

Results on pectin utilisation and flax retting ability by these organisms are recorded in Table IX, and the ret test results depicted in Figure 9.

A B C D E F G H I J K

Figure 9

Ret test results on the flax-retting ability of various organisms.

A. *Cl.felsineum* (72 hrs)
B. *Cl.aurantibutyricum* (Hf1) (72 hrs)
C. *Cl.pectinovorum* (Pj) (72 hrs)
D. *Cl.pectinovorum* var. *pseudoplectriforme* (Hf2) (120 hrs)
E. *Cl.butyricum* (Hf5) (72 hrs)

F. *C.polymyxa* (Hf4) (72 hrs)
G. *B.vulgatus mesentericus* (96 hrs)
H. *B.subtilis* (96 hrs)
I. *B.megatherium* (96 hrs)
J. *B.mesentericus* (120 hrs)
K. *B.undulatus* (120 hrs)

The two orange anaerobes *Cl. aurantibutyricum* and *Cl. felsineum* completely fermented 1 % pectin, and retted flax straws within 72 hrs at 30° C. *Cl.pectinovorum* var. *pseudoplectriforme* (Hf2) was moderately active in breaking down pectin and retted flax straws at a slower rate than the two pigmented anaerobes. *Cl. pectinovorum* (Pj) scarcely attacked pectin and showed very slight retting ability. *Cl. polymyxa* (Hf4) showed fair pectin-splitting and retting ability. *Cl. butyricum* neither attacked pectin nor retted flax straws.

Among the tested aerobic organisms, *B. mesentericus* showed moderate utilisation of pectin, and very fair activity as a retting organism. The rest of the aerobes were fair to poor in utilising pectin and in retting flax under the conditions of the experiment.

Discussion

The two chromogens *Cl. aurantibutyricum* (which is commonly distributed in our local retting liquors) and *Cl. felsineum* are beyond doubt highly active retting organisms, superior to the new plectridial anaerobe (also common in local retting liquors) which we have named *Cl. pectinovorum* var. *pseudoplectriforme*.

Strains of plectridial anaerobes found by Fribes (Winogradsky and Fribes, 1895), Stoermer (1903), Beijerinck and van Delden (1903) in Russian, German and Dutch flax-rets respectively, were claimed by their authors as the active agents of flax retting. Their choice of the specific name *pectinovorum* for this group alluded to its pectin-splitting ability. In general the retting ability of this group has, however, not been clearly established. Beijerinck and van Delden reported the weakening or final loss of retting ability of their *Granulobacter pectinovorum*. Donker (1926) found that only one of four isolates of Beijerinck's retting plectridium actually fermented pectin. Ruschmann and Bavendamm (1925) asserted that their *Plectridium pectinovorum liquefaciens* was an active retting organism, whereas Sjolander and McCoy's (1937) experience with

Ruschmann's retting organism was that it failed to utilise pectin in artificial media. In later years Ruschmann and Bartram (1943) admitted that the organism was a poor retting organism. McCoy and co-workers (1930) found that their plectridial anaerobes did not ferment pectin nor did Beijerinck's strain of *Cl. pectinovorum* which they received from the Delft collection.

Weizmann and Hellinger (1940) recorded that their strains of *Cl. pectinovorum* showed no marked ability to utilise pectin.

In short, it is interesting to note that in recent years it has become recognised that *Cl. pectinovorum* is a far less efficient retting organism than was formerly believed. Instead, the orange chromogen *Cl. felsineum* found by Carbone (1916) in Italian retting pits, has become increasingly acknowledged as the chief organism responsible for the superior retting of flax, thus confirming Carbone and co-workers' insistent claims on its superior qualities. The occurrence of this organism has now been reported from all leading flax-retting countries, as Belgium; Holland (Orla-Jensen and Kluyver, 1939), Russia (Markova, 1940; Volfson and Muratova, 1940), Germany (Ruschmann and Bartram, 1943), Australia (Lanigan, 1950) and also from N. America (McClung, 1942). *Cl. felsineum* has as yet not been found in Israel. However, using a strain of *felsineum* obtained four years ago from the American Type Culture Collection (No. 7678), we too have confirmed its excellent retting qualities.

It is an extraordinary coincidence that *Cl. aurantibutyricum* isolated in our laboratory from S. African Hibiscus stems, should prove to be a common organism in rets of locally grown flax. All orange coloured anaerobic sporulating isolates from our retting liquors were identified as this orange butyric acid-producing organism, and not the orange butanol-producing *Cl. felsineum*. Similar to *Cl. felsineum*, our original strain (W.45) and our isolates of *Cl. aurantibutyricum* from local retting liquors readily attack pectin and actively ret flax straws. It is interesting to note that Reynaud (1949) in a study on pectinolytic anaerobic bacteria, found our strain of *Cl. aurantibutyricum* to be most energetically pectinolytic compared with other pigmented clostridial anaerobes and non-pigmented plectridial anaerobes.

The comparative influence of both organisms on fibre quality and grade is being investigated in a series of laboratory experiments on flax retting under different experimental conditions.

B. polymyxa has been cited in the literature as a retting organism. From our experience it does not appear to be a retting organism of any special note.

Cl. butyricum is distinctly a non-retting organism, unable to utilise pectin and incapable of retting flax, yet this organism even in quite recent literature (sometimes cited under its synonym *B. amylobacter* (A.M.) Bredemann) has been erroneously reported as a retting organism.

TABLE VIII

*Pectin splitting ability of certain organisms on 1%
pectin medium, kept at 30°C for 72 hrs*

Organism	Pectin break-down (%)
Cl. aurantibutyricum (W.45)	100
Cl. aurantibutyricum (Hf1)	100
Cl. felsineum (A.T.C.C. 7678)	100
Cl. pectinovorum var. *pseudoplectriforme* (Hf2)	73
B. polymyxa (Hf4)	38
Cl. pectinovorum (Pj)	2
Cl. butyricum (Hf5)	1
B. mesentericus (Bc9)	77
B. megatherium (Bc5)	40
B. undulatus (Bc8) ·	19
B. subtilis (Bc4)	16
B. mesentericus vulgatus (Bc10)	7
B. mycoides (Bc7)	3

TABLE IX

The behaviour of certain organisms on sterile flax straws in water, kept at 30°C for 72 hrs

Organism	Gas (ml)	Final pH	Titr. Acidity 0.1 N acid/10 ml	Degree of Retting*
Cl.aurantibutyricum (W.45)	124	4.6	2.8	Complete
Cl.felsineum (A.T.C.C. 7678)	140	4.6	3.0	Complete
Cl.pectinovorum (Pj)	102	4.9	2.0	Slight
Cl.pectinovorum var. pseudoplectri-	91	—	—	Incomplete
forme (Hf2)	147	4.9	2.3	Complete (120 hrs)
B.polymyxa (Hf4)	52	5.0	1.2	Fair
Cl.butyricum (Hf5)	124	4.9	2.6	Nil
B.subtilis (Bc4)				Slight (96 hrs)
B.megatherium (Bc5)				Slight (96 hrs)
B.vulgatus mesentericus (Bc10)				Fair (96 hrs)
B.mesentericus (Bc9)				Very fair (96 hrs)
B.undulatus (Bc8)				Fair (120 hrs)

* See text

REFERENCES

1. ALLEN, L. A., 1946, *J. Soc. Chem. Ind. Trans.*, **65**, 373.
2. BEIJERINCK, N. W. and VAN DELDEN, A., 1904, Verhandel. Akad. Wetenschappen, Amsterdam Afdeel., *Naturkunde*, **12**, 673.
3. BERGEY's Manual of Determinative Bacteriology, 1948, 6th. ed. edited by Breed, R.S., Murray, E.G.D. and Hitchens, A.P., Williams and Wilkins Co., Baltimore, Md.
4. BREDEMANN, G., 1909, *Zentr. Bakt. Parasitenk.*, 2 Abt., **23**, 385.
5. CARBONE, D. and TOMBOLATO, A., 1917, *Staz Sper. Agr. Ital.*, **50**, 563.
6. DONKER, H. J. L., 1926, *Bijdrage tot de kennis der Boterzuur—Butylalcohol en Aceton gistingen*, Thesis, Delft.
7. DORNER, W. and RITTER, W., 1933, *Le Lait*, **13**, 366.
8. FISCHER, 1895, *Jahrb. Wissensch. Bot.*, Berlin, **27**, 1.
9. GOODWIN, L. F., 1920, *J. Amer. Chem. Soc.*, **42**, 39.
10. GRUBER, TH., 1905, *Centralbl. f. Bakt.*, Abt. **14**, 353.
11. HELLINGER, ESTHER, 1947, *J. Gen. Microb.*, **1**, 203.
12. HELLINGER, ESTHER, AVIGAN, G. and LEWIN, M., 1951, *Bull. Res. Counc. of Israel*, **1**, No. 1-2, 149.
13. HINTON, C. L., 1939, *Dept. Sci. Ind. Res. Food Invest. Special Rep. N.* 48.
14. JOHNSON, M. J., 1932, *Ind. Eng. Chem.* (Anal. ed.), **4**, 20.
15. KLUYVER, A. J., DONKER, H. J. L. and VISSER'T HOOFT, F., 1925, *Bioch. Z.*, **161**, 361.
16. KLUYVER, A. J. and VAN NIEL, C. B., 1936, *Prospects for Natural System of Classification of Bacteria*, 2 Abt., **94**, 402.
17. LANGLYKKE, A. F., PETERSON, W. H. and McCOY, ELIZABETH, 1935, *J. Bact.*, **29**, 333.
18. LANIGAN, G. W., 1950, *Nature*, **165**, 516.
19. MARKOVA, S. S., 1940, *Microbiol.* (USSR) **9**, 824 (in English 833), 1941, **35**, 7199.
20. McCLUNG, L. S., 1942, *Indiana Acad. Sci. Proc.*, **51**, 71. (Ref. in *Biol. Abst.*, 1943, **1**, 5338).
21. McCoy, ELIZABETH, FRED, E. B., PETERSON, W. H. and HASTINGS, E. G., 1930, *J. Inf. Dis.*, **46**, 118.
22. ORLA-JENSEN, A. D. and KLUYVER, A. J., 1939, *Zentralb. f. Bakt.*, (II), **101**, 257.
23. PORTER, McCLESKY, C. S. and LEVINE, M., 1937, *J. Bact.*, **33**, 163.
24. POTTER, LOUISE F. and McCoy, ELIZABETH, 1947, *J. Bact.*, **54**, 36.
25. PRAZMOWSKI, 1880, *Untersuchungen ueber die Entwicklungsgeschichte und die Formentwicklung einiger Bakterienarten*, Leipzig, p. 37.
26. REYNAUD, M., 1949, *L'Inst. Pasteur*, **77**, 434.
27. RUSCHMANN, G. and BAVENDAMM, W., 1925, *Zentr. Bakt. Parasitenk.*, 2 Abt., **64**, 340.
28. RUSCHMANN, G. and BARTRAM, H., 1943, *Z. Bakt. Parasitenk.*, 2 Abt., **105**, 326.
29. SJOLANDER, M. O. and McCoy ELIZABETH, 1937, *Zentr. Bakt. Parasitenk.*, 2 Abt., **97**, 314.
30. STOERMER, K., (a.) 1903, *Mittheil. der deutschen Landwirtschafts Gesellschaft*, **32**, 193. (b.) 1904 *Zentr. Bakt. Parasitenk.*, 2 Abt., **13**, 35.
31. VAN DER HAAR, 1920, *Monosaccharide und Aldehydsäuren*, Gebr. Borntraeger, Berlin, p. 122.
32. VOLFSON, I. A. and MURATOVA, M. A., 1940, (USSR), **9**, 672., 1941, *C. A.*, **35**, 7199.
33. WEIZMANN, CH. and HELLINGER, ESTHER, 1940, *J. Bact.*, **40**, 665.
34. WEIZMANN, CH. and HELLINGER, ESTHER, 1944, *Palestine J. Bot.*, *Rehovot series*, **4**, 51.
35. WINSLOW, C. E. A., et al. (a.) 1917, *J. Bact.*, **2**, 505. (b.) 1920, *J. Bact.*, **5**, 191.

GERMINATION INHIBITORS AND PLANT ENZYME SYSTEMS, I. CATALASE

ALEXANDRA POLJAKOFF-MAYBER

Department of Botany, Hebrew University, Jerusalem

SUMMARY: Germinating lettuce seeds show a rise in catalase activity shortly after being put into water. This rise is followed by a fall of catalase activity between the second and fourth hour of germination. It then rises again and continues to increase parallel to the progress of the germination process and the development of the seedlings.

Light increased the percentage of germination, but inhibited, *in vivo*, the catalase activity. There was no inhibition of enzyme activity by light *in vitro*.

In vitro coumarin did not affect catalase activity of lettuce seeds germinated in the light or in the dark. *In vivo*, coumarin did not prevent the formation of catalase in the seeds, although it prevented their germination.

Thiourea did not induce any change in germination percentage when conditions were favourable—in the light, but increased the percentage under unfavourable conditions—in the dark.

In vivo, thiourea inhibited catalase activity of germinating lettuce seeds. The longer the seeds were in contact with thiourea, the stronger was the inhibition of the enzyme activity.

Catalase activity failed to differentiate between seeds physiologically different but morphologically alike.

Treatments which improved the conditions of germination (light, thiourea) caused a fall in catalase activity.

Germination inhibitors, growth inhibitors and the mechanism of their action, are some of the major problems in modern physiological research. It is probable, that at least some of the stages in the mechanism of the inhibitors interfere with the enzymatic activity of the organism. Thimann and Bonner (1948, 1949a) showed that an enzyme containing a sulphydryl group is of primary importance in controlling growth, and any interference with its activity causes inhibition of growth. They also proved (1949b), that coumarin and protoanemonin interfere with such an enzyme and cause inhibition of growth in pea stems. It is of great interest to study how these substances act when inhibiting germination.

The light-sensitive variety—"Grand Rapids"—of lettuce seeds* was selected for study. They were of the 1948 crop.

Treatment of lettuce seeds with coumarin is capable of converting seeds indifferent to light into light-sensitive seeds. Thiourea is capable of the reverse action (Thompson and Kosar, 1938, 1939; Thompson and Horn 1944; Nutile 1945).

Catalase activity is very often taken as an indicator for the metabolic activity of the plant organism (Appleman 1910, 1916; Crocker and Harrington 1918; Heinicke 1923; Knott 1927: and others). We chose therefore, to investigate the correlation between coumarin, light and thiourea and the catalase activity in the germination of Grand Rapids seeds.

METHODS

Germination

Weighed amounts of seeds were germinated in Petri dishes containing one sheet of filter paper and 3 ml distilled water, or the solution to be tested. The fluid was added while illuminating with blue light (Corning Glass Filter No. 430), and the seeds were kept in darkness at 26°C for a specified length of time. For the intensity and time used, this light had no influence on germination, as proved by control experiments. In order to produce stimulation by light, 2 min of 250 f.c. from an ordinary incandescent lamp were provided after the first hour of incubation. The germination conditions were always as described above unless stated otherwise.

Catalase determination

Catalase activity was measured by estimating the volume of oxygen liberated from 10 ml H_2O_2 3% (neutralized with precipitated $CaCO_3$ to pH 6.5), by 10 ml of seed suspension. For preparing the seed suspension, 50 mg air dried seeds were germinated for various periods and then ground with 50 mg $CaCO_3$ and 10 ml distilled water. The results are expressed as the oxygen volume at 28°C, as ml 50mg/30 sec or as ml/50mg /60 sec. The suspensions were used within 5 min of their preparation.

Where seedlings of different ages had to be compared for their catalatic activity, 50 mg of dry weight (dried *in vacuo*) were used as a basis for determination.

EXPERIMENTAL

The normal course of catalase activity and the action of light

When Grand Rapids seeds were germinated under ordinary conditions and were given a light stimulus, the first roots emerged between the 18th and the 24th hour of germination. Most of the seeds germinated by the 48th hour. Those that did not germinate in 48 hours usually did not germinate at all. If the light stimulus was omitted, most of the seeds did not germinate in the first 48 hours; if then given a light stimulus, they were able to germinate to a high percentage in the following 24 hours. There was a marked difference between the catalase activity of 48 hour old seedlings and that of seeds which did not germinate in that period when both were light stimulated. But there was no difference between the catalase activity of the latter and that of seeds which did not germinate after 48 hours in darkness, many of which are capable of germination if given a light stimulus (Table I).

TABLE I

Catalase activity of seedlings and non-germinated seeds after 48 hours of germination. Calculated on basis of vacuum dry weight (ml/50 mg/30 sec).

Seedlings	Seeds failing to germinate	
	(in light)	(in darkness)
38.2	10.0	9.5
36.0	9.0	9.6

All figures are means of ten replicates.

We then measured the catalatic activity of the seeds as influenced by length of germination, with light stimulus and in darkness. The results are shown in Figure 1. The

same results were reproduced four times. From the beginning of germination (on the 18th hour), onwards the catalatic activity increased very rapidly.

In order to clarify what happens in the very first hours of germination, we measured the catalatic activity of the seeds every hour, for the first six hours of germination. The results given in Figure 2 were repeated thrice.

From Figure 2 it appears that the light inhibited the catalatic activity of the seeds. In order to clarify this point, we germinated seeds in darkness for 48 hours, selected the non germinated ones and gave half of them a light stimulus. All of them were in-
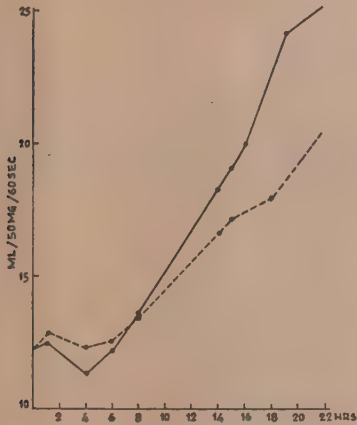
Figure 1

The curve shows the volume of oxygen per 50 mg seeds (weight before germination). Every point on the curve is a mean of four replicates. - - - - - - germination in darkness, ———— germination after light stimulus
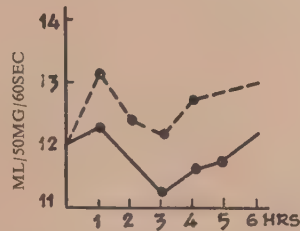
Figure 2

Catalase activity of seeds in the first hours of germination. Every point on the curve is a mean of four replicates. - - - - - - in darkness, ———— after light stimulus.
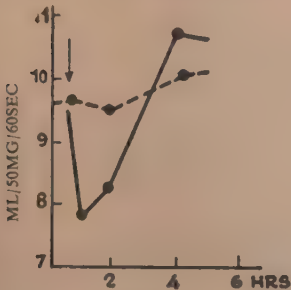
Figure 3

Catalase activity of seeds kept continuously in darkness - - - - - - and seeds which received light stimulus ————. The arrow represents the light stimulus. Every point on the curve is a mean of four replicates.
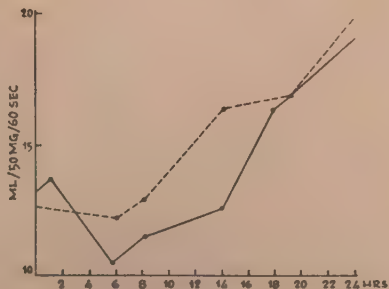
Figure 4

Catalase activity of ordinary seeds - - - - - and peeled seeds ———— germinated in darkness.

cubated another 24 hours and the catalatic activity of the two series was estimated. Figure 3 represents the results which were reproduced four times and illustrate clearly that light inhibits catalatic activity.

It is known that peeled seeds germinate to a high percentage even without light stimulus. The catalatic activity of such seeds was measured. Figure 4 summarises the results which were reproduced twice.

As seen from Figure 4, the catalatic activity of the peeled seeds is lower during the first hours of germination than that of non peeled seeds germinated under the same conditions.

Catalase activity and Coumarin

We studied the *in vitro* action of coumarin on the catalase activity of the seeds germinated under various conditions of illumination. For this purpose seeds were germinated in darkness, in continuous red light (Corning glass filter No. 435) and after receiving a light stimulus of 250 f.c. for 2 min. The suspension of catalase was prepared in coumarin (10 mg%), the controls being suspended in water. The results are summarised in Table II.

TABLE II

The in vitro *action of coumarin on catalase activity of seeds germinated under various conditions of illumination (calculated on the basis of weight before germination).*

Light condition	Germination %	Oxygen liberated in ml/50 mg 30 sec		Probability
		Test	Control	
Darkness	17	14.8	14.0	0.6 —0.5
,,	19	18.4	17.8	0.6 —0.5
Red light	93	44.1	46.4	0.7 —0.6
,, ,,	97	49.3	49.3	
,, ,,	94	42.2	43.1	0.7 —0.6
Incandescent light	96	48.1	42.9	0.05—0.01
,, ,,	97	44.5	43.7	0.6 —0.5
,, ,,	95	42.4	41.7	0.6 —0.5

All figures are means of ten replicates

As indicated by experiment No. 6 (Table II), it might be that coumarin is able to stimulate catalatic activity of seeds germinated under ordinary light conditions. We therefore investigated its action *in vitro* using a higher concentration (16 mg%). (See Table III).

These experiments show that coumarin *in vitro*, did not affect catalase activity. When germinated in coumarin (4 mg%—a concentration which does not inhibit germination more than 50%), catalase formation was not inhibited, as illustrated in Figure 5. The curve of catalatic activity rises with prolongation of germination time. In Figure 5 we also see that seeds germinated in water and darkness show higher catalase activity then seeds germinated in coumarin and darkness, probably because of the higher percentage of germination in the former. Figure 5 illustrates once more the inhibiting action of light on catalase activity *in vivo*.

TABLE III

The in vitro action of coumarin on catalase activity of seeds germinated under ordinary light conditions

| Germination % | Oxygen liberated in ml/50 mg/30 sec | | Prob-ability |
	Test	Control	
95	44.2	44.6	0.5—0.4
100	53.4	52.5	0.5—0.4

All figures are means of ten replicates.

TABLE IV

The in vitro action of thiourea on catalase activity of lettuce seeds

| Germination % | Oxygen liberated in ml/50 mg/30 sec | | Prob-ability |
	Test	Control	
95	29.7	28.5	0.4—0.3
93	23.9	26.1	0.1—0.05

All figures are means of ten replicates.

Catalase activity and thiourea

Thiourea, which is frequently used as a dormancy breaking substance, neither enhanced nor inhibited the catalase activity of lettuce seeds *in vitro*. Table IV represents the results of the *in vitro* action of thiourea (125 mg%) on catalase activity of seeds germinated for 24 hours under ordinary conditions with light stimulus.

According to Denny et al. (1930), thiourea inhibits catalase from potato tubers *in vitro* only when its concentration exceeds 150 mg%. We worked with lower concentration and did not try to raise it, because of the very marked inhibitory effect exerted by this low concentration on catalase activity *in vivo*, as seen from Table V.

This inhibitory action of thiourea was illustrated even more clearly in the following experiment. Seeds were germinated in water and darkness for 48 hours, when the non-germinated ones were separated; half of them were transferred to thiourea 125 mg%, the other half into fresh water. All the manipulations were carried out in blue light. The catalatic activity was estimated after various lengths of time. The results are summarised in Figure 6 and were reproduced thrice. The germination percentage was 2% in water and 24% in thiourea.
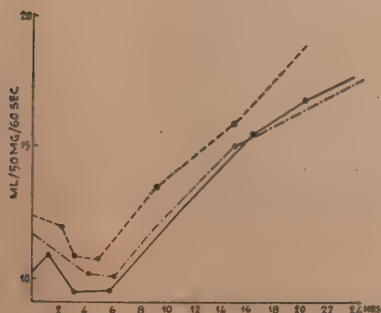
Figure 5

Catalase activity of seeds geminated: in water in darkness - - - - - -; in coumarin in darkness -.-.-.-.-.-; in coumarin in light ————.
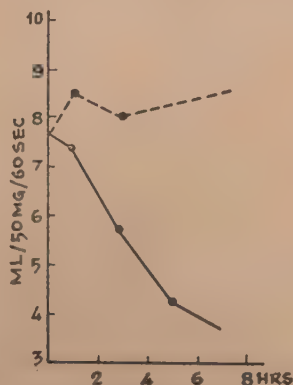
Figure 6

The *in vivo* effect of thiourea on catalase activity of lettuce seeds. - - - - - - seeds in water, ————seeds in thiourea. Every point on the curve is a mean of four replicates.

TABLE V

Catalase activity of seeds germinated in thiourea 125 *mg%.   The controls germinated in water.*

Germination conditions	Test		Control	
	Germination %	Oxygen ml/50 mg/60 sec	Germination %	Oxygen ml/50 mg/60 sec
Light	95	13.8	93	42.1
„	95	11.9	94	44.0
Darkness	22	6.0	17	30.1

All figures are means of ten replicates

DISCUSSION

There was no difference in appearance between the seeds which did not germinate after the first 48 hours of germination, having got a light stimulus and those which did not germinate but had been kept in darkness. There was, however, a very marked physiological difference. The latter may germinate to a high percentage later on, if given a light stimulus while the former will not. This physiological difference did not show in their catalatic activity (Table I).

The catalatic activity of the seeds increased with increasing % of germination and development of the seedlings. The curve of catalase activity during germination (Figure 1) showed that this increase was not continuous. There was first a decrease of activity, in the initial stages of germination, followed by the continuous rise. This corresponds to the observations of Rhine (1924) and of Rangan and Mallik (1931). Still more careful study of the first hours of germination reveals that there actually is a very slight rise in catalase activity immediately after imbibition of water by the seeds. This rise although statistically non-significant, was generally reccurring. It was followed by a fall of activity between the second and fifth hours of germination, after which the continuous rise began. This course of activity was typical for seeds germinated after a light stimulus and of those germinated in darkness. In seeds germinated with light stimulus, however, the fall of catalatic activity was more marked and was kept at a lower level until the 8th or 10th hour, when it rose rapidly and exceeded the catalatic activity of the seeds germinated in darkness (Figure 1 and 2). The question arose, whether the light inhibited catalase activity. Experiments summarised in Figure 3 point to the possibility that light either destroys catalase or inhibits its action, as the seeds which received light stimulus should have had at least as much catalase as those which remained in darkness. A similar fact was observed by Eyster (1950), who found that corn plants which grew in light had lower catalatic activity than those grown in darkness.

When germination was improved by other means, such as peeling (Figure 4) or thiourea treatment (Figure 6), the result was again a lower catalase activity. This points to the fact that in the early stages of germination, favourable germination conditions are accompanied by low catalse activity in the imbibed seeds.

Coumarin, *in vitro*, did not inhibit the catalase from lettuce seeds germinated in the dark, although, *in vivo* the same concentration of coumarin (10 mg%) caused the complete inhibition of germination. Coumarin did not inhibit the catalase from seeds

germinated in red light—which is the most effective in stimulating germination—or those germinated after receiving an ordinary light stimulus (Tables II and III). Therefore the mechanism of germination inhibition by coumarin is not by blocking the catalase activity, nor does it interfere with catalase formation in the seed, as is illustrated in Figure 5, where the concentration of coumarin used (4 mg%), still allowed germinatiom.

Thiourea, as a dormancy breaking substance, was capable of improving germination under unfavourable conditions (darkness—Table V—and germination data for Figure 6), but had no effect at all under favourable conditions (light—Table V). Thiourea, *in vitro*, had no effect on catalase activity of the lettuce seeds (Table IV), but inhibited the catalase *in vivo* (when germinated in thiourea—Table V). The longer the seeds were in contact with thiourea, the stronger was the inhibition (Figure 6). This may be due to the destruction of the enzyme, a supposition which remains to be proved experimentally.

The observations made here show a substance which, while improving germination, strongly inhibits catalase activity. As was already stated, catalase activity failed to differentiate between seeds physiologically different but morphologically alike (Table I). Therefore catalase activity cannot here be taken as a reliable indicator of metabolic activity.

Keilin and Hartree (1945) assume that catalase *in vivo*, acts as a peroxidase. In the light of the described effect of thiourea on catalase activity, it would be most interesting to investigate the course of activity of other peroxidases or any other enzymes having an iron porphyrin group in their structure, following thiourea treatment.

REFERENCES

1. APPLEMAN, CH. O., 1910, *Bot. Gaz.*, **50**, 182.
2. APPLEMAN, CH. O., 1916, *Amer. J. Bot.*, **3**, 223.
3. CROCKER, W. and HARRINGTON, T., 1918, *J. Agr. Res.*, **15**, 137.
4. DENNY F. E., MILLER, L. P. and GUTHRIF, J. D., 1930, *Contr. Boyce Thomps. Inst.*, **2**, 417.
5. EYSTER, H. C., 1950, *Plant Physiol.*, **25**, 630.
6. HEINICKE, A. J., 1923, *Cornell Univ. Agr. Exp. St. Memoir*, **62**, 9.
7. KEILIN, D. and HARTREE, F. E., 1945, *Biochem. J.*, **39**, 293.
8. KNOTT, J. E., 1927, *Cornell Univ. Agr. Exp. St. Memoir*, **106**, 1.
9. NUTILE, G. E., 1945, *Plant Physiol.*, **20**, 433.
10. RHINE, L. E., 1924, *Bot. Gaz.*, **78**, 46.
11. RANGAN, J. and MALLIK, A. K., 1931, *New Phyt.*, **30**, 355.
12. THIMANN, K. V. and BONNER, W. D., 1948, *Amer. J. Bot.*, **35**, 271.
13. THIMANN, K. V. and BONNER, W. D., 1949a, *Amer. J. Bot.*, **36**, 214.
14. THIMANN, K. V. and BONNER, W. D., 1949b, *Proc. Nat. Acad. Sci.* (U.S.), **35**, 272.
15. THOMPSON, R. C. and HORN, N. L., 1944, *Proc. Amer. Soc. Hort. Sci.*, **45**, 431.
16. THOMPSON, R. C. and KOSAR, W. F., 1938, *Science*, **87**, 218.
17. THOMPSON, R. C. and KOSAR, W. F., 1939, *Plant Physiol.*, **14**, 567.

SILURIAN IN THE NEGEV (ISRAEL*)

L. PICARD

Geological Institute, Government of Israel and the Hebrew University, Jerusalem

SUMMARY: On the basis of the brachiopod genus *Platystrophia*, a Silurian age was determined for the much-discussed marine intercalations occurring in the lower part of the Nubian Sandstone series north of Elath (Negev).

In our discussion (1943, pp. 30—33) on the Paleozoics of the Arabian Mainland, stress was laid on the epicontinental shallow-water character of the marine sediments which are found as intercalations in the "Red-beds" complex of the Nubian Sandstone. Sporadic outcrops of Cambrian in the Dead Sea region, of Ordovician and Gothlandian in Inner-Arabia, of Carboniferous in Egypt and Sinai were rare witnesses of these transgressions. We wrote at that time (p. 31), "It must be never forgotten that in the course of such vast periods of time, the frequent denudation, deposition and re-deposition of the Nubian Sandstone has often destroyed and removed the original interbedded marine layers". Although we "assumed that Palestine and Transjordan were situated in the vicinity of the coast during the Paleozoic" we granted the Silurian sea "an exception, leaving, as it did, graptolite-bearing shales in Central Arabia" in spite of the fact that "up till now this formation has not been discovered in our western countries". This concept was also demonstrated in a diagram (Figure 6, p. 33) in which the Gothlandian transgression penetrated nearly as far inland as in a later period did the Cretaceous transgression.

The only outcropping Paleozoic rocks in the southern-most Negev which are geographically connected with the sedimentary Sinai (1951) are those of the Wadi Menaiye situated 30 km northwest of the Gulf of Akaba and on the Western border of the Araba-graben. Sections of this locality were published by Blake in 1936 (p. 75, 76) which recorded *Obolus* and *Siphonotreta* determined by Leslie R. Cox of the British Museum and referred to by Blake as Cambrian. The fossils were found in black limestone beds intercalated within the Nubian Sandstone complex. A short visit in the spring of 1936 revealed to us the facial relationship which exists between this Paleozoic section and that of Wadi Hasa on the southeast corner of the Dead Sea (Transjordan), and also brought to our mind related sections drawn and described by Ball (1916) from the manganese-bearing deposits of Umm Bogma in Western Sinai. Ball assigned to the Umm Bogma beds a Carboniferous age. Similar manganese-bearing beds were found at Menaiye lying above the "Cambrian" limestone which Blake—in analogy with Umm Bogma—referred to the Carboniferous.

At Menaiye, as at Umm Bogma, the lower part of the Nubian sandstone (Hull's desert sandstone of the Sinai region) rests unconformably upon the crystalline Precambrian. This Lower Nubian Sandstone consists principally of black and wine-red, often coarse, arkosic- and gravel-bearing sandstone. But in Sinai, it also contains sandy

shales and marls with Problematica. They were drawn and described by Barthoux (1922) and were compared by us (1942) with *Cruziana* (*Bilobites*). The Lower Sandstone of Menaiye which varies in thickness from 30 to 100 and more metres, is followed by 10 metres of well-bedded dark limestone and dolomite from which Blake collected the "Cambrian" fossils. Above the limestone series is a 25 metres series of sands, shales and banded clays (the main bearer of manganese). The laminated and manganiferous series are overlain by 100 metres of multicoloured sandstone which resembles and is most likely contemporaneous with the Paleo-Mesozoic Arnon-Petra Sandstone of Transjordan. On both sides of the Rift Valley, this colourful sandstone is capped by a uniform, white sandstone, 250 m thick at Menaiye (Blake, 1936), which thus takes the stratigraphic position of Kober's Homejma Sandstone. (The Homejma sandstone is probably identical with the Ram sandstone of Quennel, ascribed by this author, however, to the Cambrian). In Transjordan, the white sandstone of Homejma is followed again by variegated—though prevalent violet sandstone.

In Cis- and Transjordan, Lebanon and Syria it frequently forms the terminating part of the Nubian Sandstone complex and is assigned to the Lower Cretaceous by well-defined plant remains.

The section of Menaiye is thus divided into: Lower Nubian Sandstone, Limestone horizon, shale-clay-ore series and Upper or Main Nubian Sandstone (with its subdivisions). The section differs from that in the Sinai in two major points:

(1) The shale-clay-ore series occur in the Sinai at the base of the limestone beds.

(2) The limestone beds in the Sinai outcrops (Hull's Nasb limestone) are defined by distinct Carboniferous fossils, whereas in the Negev at Menaiye the few brachiopods were attached to the Cambrian.

Shaw (1947) very recently tried to solve this apparent discrepancy which exists between the sections of both localities. He measured in greater detail the section of Menaiye below the Main Nubian Sandstone, i.e. ore beds, limestone and Lower Nubian Sandstone. But as no new fossils were discovered the question of age, particularly of the dark limestone beds, remained unsolved, and Shaw leaves us with the following alternatives: either (1) Carboniferous and Cambrian or (2) only Carboniferous or (3) only Cambrian is present in the rock series.

In connection with groundwater research, in February 1950, the writer again made a brief visit to the Menaiye region. He found in the dark limestone beds, among other fossil remains, a well preserved articulate brachiopod which at a first glance was thought to be a *Productidae*. As, however, no literature was at hand—our departmental library at Mount Scopus still being cut off from town—the writer used the opportunity of being present in Algier during a Unesco meeting in spring 1951, to hand over the fossil to Prof. H. Termier of the Faculté des Sciences for determination. Prof. Termier soon recognized the form as *Platystrophia* sp. and drew our attention to Schuchert and Cooper's work (1932, 1949, p. 299) on this genus. According to Cooper, *Platystrophia* existed from the Middle Ordovician to the Middle Gothlandian ("Silurian" of N. American stratigraphers).

The findings of distinct Silurian fossils leads to the following conclusions:

(1) It confirms our concept of a major Silurian transgression on the Arabian Shield.

(2) It rejects the idea of a Carboniferous age for the Menaiye limestone and favours

the assumption of a Lower Paleozoic age for most of the strata below the main man-
ganese-ore horizons.

(3)   At Umm Bogma in the Sinai most of the strata below the Carboniferous Lime-
stone (Hull's Nasb Limestone), which seem to correspond to our Lower Nubian Sand-
stone, are again likely to be of the same Lower Paleozoic age.

(4)   The earlier determination of *Obolus* in the limestone beds of Menaiye may
belong to the Silurian age. The determination of *Siphonotreta*, a brachiopod occurring
in the Cambrian, needs re-examination.

(5)   It seems questionable to refer all the beds of the main manganese ore deposits
of Umm Bogma (Sinai), of Menaiye (Negev) and of Dana (Transjordan) to the Car-
boniferous; they may as well belong to the Lower Paleozoic. This belief is strengthened
by our observations (1941) of manganese concretions in the base layers of the Cambrian
marine limestone of Wadi Hasa (Transjordan).

(6)   Our survey in Wadi Hasa and the foregone results obtained from Wadi Menaiye
justify the assumption that the rock series of Transjordan (including Dana) and the
Negev, hitherto regarded exclusively as Cambrian or as Carboniferous, represent
formations varying in age from Uppermost Lower Cambrian to Silurian. No definite
Carboniferous rocks have yet been established there.

REFERENCES

1.   BLAKE, G. S., 1936, *The Stratigraphy of Palestine and its Building Stones*, Jerusalem.
2.   BLAKE, G. S., 1940, in IONIDES M. G., *Report on the Water resources of Transjordan*, London.
3.   BALL, J., 1916, *The Geography and Geology of West Central Sinai*, Cairo.
4.   BARTHOUX, J., 1922, Chronologie et description des roches ignées du desert arabique, *Mem. Inst.
     Egypt.*, Vol. V.
5.   COOPER, G. A., 1949, in Shimer, H. W. and Shrock, R. R., *Index Fossils of North America.*
6.   HULL, E., 1886, Memoirs on the Geology and Geography of Palestine. Survey of Western Palestine,
     Palestine Exploration Fund, London.
7.   KOBER, L., 1919, Das noerdliche Hegaz, *Denkschr. math. nat. kl. Ak. Wiss.*, Wien, **91**.
8.   PICARD, L., 1942, New Cambrian Fossils and Paleozoic Problematica from the Dead Sea and
     Arabia, *Bull, Geol. Dep. Hebr. Univ.*, **4**, No. 1.
9.   PICARD, L., 1943, Structure and Evolution of Palestine, *Bull. Geol. Dept. Heb. Univ.*, **4**, No. 2-3-4
10. PICARD, L., 1951, Geomorphogeny of Israel. Part I. The Negev. Geol. Survey of Israel. Publica-
     tion No. 1. Reprint from *Bull. Res. Counc. of Israel*, **1**, No. 1—2, **5**.
11. QUENNEL, A. H., 1951, The Geology and Mineral Resources of Transjordan, *Colonial Geology
     and Mineral Resources*, **2**, No. 2, London.
12. SCHUCHERT, C. and COOPER, G. A., 1932, Ortheidea and Pentameroidea. Peabody Museum.
     Vol. IV, Part I.
13. SHAW, S. H., 1947, Southern Palestine Geological Map 1:250,000, with explanatory notes, Jeru-
     salem.

OBSERVATIONS ON THE BEHAVIOUR AND THE EGG DEVELOPMENT OF *TMETHIS PULCHRIPENNIS ASIATICUS* UV.

A. SHULOV

Hebrew University, Jerusalem

SUMMARY: Mating habits, oviposition and digging in behaviour of *Tmethis pulchripennis asiaticus* Uv. are described. The seasonal cycle of development was studied. It was found that eggs do not imbibe any water during their development.

INTRODUCTORY

The present observations on the behaviour and egg development of *Tmethis pulchripennis asiaticus* Uv. were carried out in the course of an investigation of Krauss' organ in this grasshopper (Shulov, 1952) during three breeding seasons in 1949, 1950 and 1951.

Hoppers and adults of *Tmethis pulchripennis asiaticus* Uv. were collected in Jerusalem in the spring. The insects were identified according to Uvarov's key (1943). The eggs used for the following experiment were laid in the laboratory.

GEOGRAPHICAL DISTRIBUTION

This grasshopper is found in the countries bordering on the eastern Mediterranean (Israel, Transjordan, Syria, South Turkey), Iraq and Persia (Uvarov, 1943). It inhabits open country on bare soil on areas covered with sparse vegetation and on stony ground (Uvarov, l.c.), Chopard (1938). Its colours vary widely with the environment, a feature common to many other geophilous Acrididae. Its resemblance to ground is sometimes striking. On the white limestone rocks of Judea they are ashen white. On other slopes of the same hills with their brown terra rosa and in the moulds between the hills they are brownish. In Galilee on soils of basalt origin, they are dark grey, almost black. The hatching hoppers are mostly brownish, and their bright grey patterns vary considerably among individuals emerging from the same egg-pod.

THE ANNUAL CYCLE

Adults are found in May and June. Oviposition takes place during late May and June. The young hoppers of the first instar are found from the end of October through February. (Bodenheimer 1935). Active development of the hoppers takes place from February through May. In the laboratory they hatch during October and early November. Apparently they pass the winter as first instar nymphs.

COURTING AND MATING

The male grasshopper follows the female and strikes her with his antennae. Sometimes he approaches from behind, and a sharp thrust of the female's hind leg propels him

like a ball to a considerable distance. When the female ceases to crawl about and re-
mains quiescent, the male approaches from the side, retreats a little and hammers her
thorax with his head. This hammering is repeated several times until mating starts.

Mating proceeds in the usual way. The male jumps on to the back of the female,
projects his abdomen downwards and applies his genitalia to the posterior end of the
female's abdomen. The male simultaneously lifts both his hind legs and forcibly strikes
the female's back and legs. His antennae, fore and mid legs tremble violently as does
his abdomen to a lesser extent. This procedure is repeated several times.

Copulation may continue for eight hours and more. It was observed at a temperature
range of 26°C—32°C.

<center>OVIPOSITION</center>

The female lays her eggs in hard, dry soil. She starts burrowing after having explored
the oviposition site with her widely open genital apophyses. The digging in of the ab-
domen continues for from 5 to 15 minutes. Expulsion of the eggs is associated with
repeated protrusion and retraction of the head.

After approximately twenty minutes the female starts withdrawing her abdomen
from the soil. Simultaneously the hind legs begin to beat the soil around the abdomen
and to draw dust and sand toward it. Five to ten such "collecting" movements of the
legs are followed by one pressing movement directed downwards against the soil be-
neath the abdomen. The abdomen is thus slowly withdrawn and the soil is pressed
around it in such a way that when its posterior end reaches the soil surface, the hole
is already filled with pressed earth and sand. The collecting and pressing of loose earth
continues for an additional few minutes after the abdomen has been withdrawn. The
female then crawls to a distance of several centimetres from the site of oviposition,
while the described movements of the hind feet gradually cease.

In the laboratory an average of 4 egg-pods per female were laid by 10 females.

<center>THE EGG-PODS AND THE EGGS</center>

Tmethis pulchripennis asiaticus Uv. lay their eggs into dry, hard ground. The egg-pod
is shaped like a long-necked bottle (Figure 1B). The length of the "neck' varies with
the depth of the hole which the ovipositor was able to burrow. The range is from 4 to

Figure 1

Egg-pods of *Tmethis pulchripennis asiaticus*
Uv. showing variation in shape. A—section,
B—external view.

26 mm. The width of the neck is about 5 mm. The neck is elongated, narrow and slightly curved, and is filled with a frothy substance which also surrounds the eggs and serves as a bolster towards the bottom of the egg-pod. When fully formed, the "neck" is covered by a small concave lid. The "neck" is sometimes partly filled with eggs. The lower part of the egg-pod varies in shape depending on the conditions of the soil and the number of eggs (Figure 1A). The lowest eggs are sometimes glued to a small stone which impeded further digging. The dimensions of the eggs are given in Table I.

TABLE I

Measurements of the eggs during their development

Days after oviposition	14	42	60	111	124
Length (mm)	6.5 (13)	5.7 (10)	5.9 (4)	5.7 (9)	6.1 (18)
Width (mm)	1.8 (13)	1.7 (10)	1.9 (4)	1.8 (9)	1.8 (18)

The number of eggs in an egg-pod varies considerably. Among 46 egg-pods studied there were 8 eggs in the smallest and 37 in the largest (average 16.4). The freshly laid eggs are large and heavy in comparison with those of other locusts and grasshoppers which imbibe water during their development. Table II presents the comparative weights of eggs of some grasshoppers close to oviposition, at the end of anatrepsis and before hatching.

TABLE II

Changes in weight of eggs of some grasshoppers during their development

Species	Weight in mg and the number examined (in brackets)			Author
	Shortly after oviposition	At the end of anatrepsis	Before hatching	
Anacridium aegyptium L.	6.6 (54)	13.5 (87)	14.6 (21)	Shulov (in preparation)
Dociostaurus maroccanus Thnb.	6.5—7(150)	5.5 (50)	9.4 (50)	Bodenheimer-Shulov (1951)
Melanoplus bivittatus Say	4.16 (128)	6.69 (128)	?	Salt (1949)
Schistocerca gregaria Forsk.	10.5 (30)	19.1 (30)	23.5 (37)	Shulov (in press)
Tmethis pulchripennis asiaticus Uv.	13.4 (30)	13.1 (19)	8—13(41)	

THE DIGGING IN

Hoppers of *Tmethis* have been observed in the laboratory to dig themselves into the soil when the temperature dropped below 13—15°C. They commence scratching the surface with their hind legs, then lie down flat with the body pressed towards the soil. They then begin to swing the body from side to side shoveling away the earth.

Uvarov (1928) cites Nikolsky's (1925) observations on *Acrotylus insubricus* in Turkestan which buries itself in the soil when the temperature drops below 10°C. This

procedure occurs in autumn every night, but later in the season it ceases to emerge and remains buried until the spring. This behaviour is similar to that observed in *Tmethis*' young hoppers which pass the cold winter days buried in the soil as actually observed in the laboratory.

EGG DEVELOPMENT

Eggs ready for oviposition, removed from the abdomen of the female, weigh 13.5 mg (average of 103), the range being 9.8—16.3 mg. On the day of oviposition, their weight is 12.4 (average of 51 eggs). Thus there is no considerable change in the weight of the eggs from oviposition to eclosion and certainly no increase. Eggs were observed which weigh only 8—9 mg before successful hatching. Figure 2 presents weights of 188 eggs removed from the egg-pods at various stages of their development.

Preliminary experiments during the summer of 1949 showed that only those eggs which remain continually in the dry sand are able to develop. Two series of experiments were set up: A. Incubation in dry sand. B. Incubation initially in dry sand and later transferring to contact with water.

A. Egg-pods were placed in small glass jars filled with dry sand. Two samples of sand were weighed, dried for 6 hours at 110°C and then reweighed. The percentage of moisture found was 0.25—0.35% in both samples.

The jars were kept at the temperature of 25—26°C. The egg-pods were taken out at fixed times, opened, and the eggs were measured and weighed; then embryos were examined using the excellent method of Slifer (1945). Table III presents these results.

The eggs are laid at the end of May and in June. They start developing late in July

Figure 2
The changes in weight during development of 188 eggs, without water.

and continue through August and September. The main period of hatching in the laboratory was at the end of October. The only "diapause" if it can be called so, is before the beginning of development.

B. Nineteen egg-pods were placed as above in small glass jars filled with dry sand. The egg-pods were then transferred to moist sand. Two to three eggs were examined at intervals. Table IV shows that practically no successful development is possible in contact with water. The control egg-pods placed in dry sand showed a high percentage of hatching.

Eggs removed from the egg-pods during experiment A were placed on slightly moistened plaster of Paris, but only a few of them continued to develop.

TABLE III

Development of the eggs in the dry sand

Month	VI	VII			VIII			IX			X			XI	
Degrees after Bod.-Shulov (1951) — Days from oviposition	1-9	10 19	20 29	30 39	40 49	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149
Degrees of development															
0 — No conspicuous germ. band	16	38	24		7	15	29				2	5			
VIII — Until the abdominal appendages appear		4	25	3	5	1		3							
X — Until the thoracic legs fold ventro-mediad				5	3	4	6	8	1				3		
XIV — Until the end of anatrepsis						2		3	1	2			5		
XX — Ready for hatching												16			
Hatching											7	2	58	32	3

TABLE IV

The effect of transferring egg-pods into contact with water at various times

No. of experiment	After how many days transferred	Stage after Bod.-Shulov	Stage of development when transferred	No. of eggs in pod	Results; No. of eggs hatched
15	14	0—1	Triangular embyo— no differentiation	14	0
14	21		"	17	0
12	15		"	17	0
1	27		"	16	0
2	31	IV	Thoracal appendages appear	14	1
16	33		"	8	0
17	34		"	13	0
3	35	VII	Abdominal appendages appear	20	1
18	45	X—XI	Maxillary palps distinct	12	0
4	51		"	18	0
19	56		"	?	0
13	67		"	14'	2
5	72		"	13	0
6	92	XIII—XIV	The antennae reach the fore legs End of anatrepsis	24	0
7	Control			19	7
8	"			26	21
9	"			12	7
10	"			18	16
11	"			23	21

PARASITES

A couple of parasitic flies bred from *Tmethis* adults collected in the field were identified by Dr. F. van Emden of the Commonwealth Institute of Entomology as *Acemyia uncinata* Thoms. (Tachinidae). The bodies of dead grasshoppers have been investigated and several Tachinid larvae have been found in them. The maximal number of larvae observed in one grasshopper was five.

DISCUSSION

Three interesting features concerning mating, digging in and oviposition of *Tmethis pulchripennis asiaticus* Uv. have been described. Digging in, which was described by Nikolsky (1925) for *Acrotylus insubricus*, is associated in *Tmethis* with hibernation in the larval stage.

Preliminary observations on the eggs of Tmethis have shown that they develop in the absence of water. Incubation of eggs in dry sand and in contact with water confirmed the conclusion that only those eggs develop which have been kept without any access to water. This feature is peculiar and not yet known in any grasshoppers (Prof. Uvarov in personal communication). The eggs which develop without diapause (Schistocerca, Anacridium, Locusta) as well as those with a diapause (Melanoplus, Dociostaurus, Austroicetes) imbibe water during their active development. The type of egg development described for *Tmethis pulchripennis asiaticus* seems to be connected with the distribution of this species in regions with practically no summer rains.

The eggs are laid late in May and in June and their development continues for about three and a half months at a temperature of 25°C. Hatching in the laboratory coincided with this period of hatching in the field in Jerusalem. The young hoppers pass the winter in a condition of semitorpor, feeding on sunny days. Active development starts in February. The adults appear in May.

There is a certain stage, just after oviposition, which might be called a diapause. It continues from one to one and a half months, and is similar to the diapause found in *Dociostaurus maroccanus* (Bodenheimer and Shulov, 1951). No diapause seems to occur at the stage of late anatrepsis found in *Dociostaurus maroccanus* Thnb. (Bodenheimer and Shulov, 1951), *Melanoplus differentialis* Thos. (Slifer, 1932), *Melanoplus bivittatus* Say, *Melanoplus mexicanus mexicanus* Sauss., *Melanoplus packardi* Scudder (Salt, 1949a).

ACKNOWLEDGEMENT

REFERENCES

1. BODENHEIMER, F. S., 1935, *Animal Life in Palestine.*
2. BODENHEIMER, F. S. and SHULOV, A., 1951, *Bull. Res. Counc. of Israel,* **1**, No. 1—2, 59.
3. CHOPARD, L., 1938, *La biologie des Orthopteres,* Paris, *Encycl. Ent.*
4. NIKOLSKY, V. V., 1925, *The Asiatic locust,* Leningrad.
5. SALT, R. W., 1949, *Canad. J. Res.,* **D27**, 233.
6. SALT, R. W., 1949a, *Canad. J. Res.,* **D27**, 236.
7. SHULOV, A., 1952, *Bull. Ent. Res,* **43**, 469.
8. SHULOV, A., "An induced interruption of development in eggs of *Anacridium aegypticum L.*" (in preparation).
9. SHULOV, A., 1952, *Bull. Res. Counc. of Israel,* **2**, 58.
10. SLIFER, E. H., 1932, *J. Morph.,* **53**, 1.
11. SLIFER, E. H., 1945, *Science,* **102**, 282.
12. UVAROV, B. P., 1928, *Locusts and grasshoppers,* London.
13. UVAROV, B. P., 1943, *Trans. R. Ent. Soc. Lond.,* **93**, 1.

AN OCCURRENCE OF TERRA UMBRA NEAR ROSH HA'AYIN

A. LOEHNBERG and E. LOEHNBERG

Kfar Shmaryahu

SUMMARY : A subsurface occurrence of terra umbra in a boring near Rosh Ha'ayin is reported.

Bore-hole samples from Rosh Ha'ayin revealed terra umbra, a brown natural earth pigment at a depth of 32.5—37.3 m. Geological and physical properties of this mineral were examined, and its occurrence, origin and the possibility of its exploitation were investigated*. Attempts have been made to correlate its stratigraphic and physical properties with the results of surface geolectric prospecting.

THE DISCOVERY BORE

Coordinates: 144510—167130. Elevation: 33.6 m above sea level.

Log :

0.0— 0.3 m	dark grey to brown loamy soil
0.3— 6.5 m	brown heavy loam containing breccie of limestone and flint
6.5—14.5 m	limestone breccie embedded in brown loam
14.5—16.0 m	limestone and flint breccie with less loam
16.0—18.0 m	do. with chalky "nari" and little loam
18.0—21.0 m	whitish and yellowish chalky and sandy "nari"
21.0—22.0 m	limestone boulders and breccie
22.0—23.5 m	greyish-whitish-yellowish limestone with flint (first water-bearing formation; water rose to 11 m below ground)
23.5—28.0 m	white porous somewhat chalky fossiliferous limestone
28.0—32.5 m	breccie and boulders of crystalline limestone
32.5—37.2 m	dark-brown soft earthy material (terra umbra)
37.2—40.0 m	limestone and flint boulders and breccie embedded in brown loam
40.0—45.0 m	hard limestone and flint conglomerate
45.0—53.0 m	white porous shelly fossiliferous limestone
53.0—58.0 m	do., slightly chalky
58.0—69.5 m	white porous fossiliferous limestone
69.5—69.9 m	limestone containing loamy and clayey material in fissures

The samples between 53.0—58.0 m and 69.4—69.8 m are described by the Mandatory Government Geologist S. H. Shaw as follows:

53.0—58.0 m	white chalky fossiliferous limestone resembling meleke (rudists present or corals); compare Miocene coral limestone from Deir Tarif.
69.4—69.8 m	white, red soil-stained chalk (no forams)

Thin sections, made from these two samples, were examined by M. Avnimelech of the Hebrew University, who according to the informations of Mr. Shaw, has reported as follows:

Both sections reveal very little as the material is calcified and porous. They are

practically identical, both of them being a soft white limestone made entirely of fragments of bivalves (perhaps Ostreids). In the first (higher) sample one very badly visible fragment may belong to Orbitolina. Many sections of echinoid-radioles are present as well. In the second slide I found several 'Astacolus'-like forams and a 'Dentalina'. Radioles of Echinoids were also found.

As the determinations are questionable the conclusions are uncertain. Nevertheless, the character of the rock, together with its faunal remnants, point to Upper Cenomanian or to Turonian.

UMBER

A representative sample of the 4.8 m section of umber penetrated between 32.5—37.3 m depth gave the following chemical composition:

	100° C (%)	300°C (%)
Moisture	11.0	∼ 1
Loss on ignition	22.0	∼ 24
MnO_2	12.0	∼ 13
Fe_2O_3	51.1	∼ 56
Other constituents	3.9	∼ 6

For comparison two analyses of high quality foreign umbers are cited:

	Cyprus (%)	Derbyshire (%)
Moisture	4.8	5.2
Loss on ignition	8.8	—
MnO_2	19.0	11.5
Fe_2O_3	48.5	22.5

Pigmentation tests (R. Larisch*) indicated a very good quality and colouring power. Shades could be produced by treating the raw umber under different temperatures of heating.

GEOELECTRIC SURVEYING

In order to find out whether the umber would give electric indications on geophysical surface measurements at the discovery site and in its environment, resistivity surveys were carried out in 1943, 1945 and 1947. Field measurements were made according to the standard 4-point electrode arrangement with measuring intervals taken at 2.5 m changes of the electrode position to depths of 70 m, and at 5 m intervals beyond 70 m penetration depth. The field diagrams of apparent resistivities as dependent on the penetration depth of an electric current were subjected to mathematicgraphic treatment (Loehnberg and Loewenstein, 1936), so that at each site tested, a log of absolute specific resistivities could be obtained.

Figure 1 shows the field diagram at the bore, the log of calculated (true) resistivities and the main formations penetrated. A correlation between geoelectric and bore-hole data follows:

* Consulting Industrial Chemist, Tel Aviv.

calculated resistivities
התנגדויות המחושבות
relative resistivity (Ω-cm) התנגדות יחסית

| Bore בורה | Ω.cm 0 | 500 | 1000 | 1500 | 2000 | 2500 | 3000 | 3500 |

LOAM, BRECCIE, חרסית וחלוקים

COVER LAYER שכבת כסוי

INTERMEDIATE LAYER גוש ביניים

BOULDERS, LIMEST CONGL חלוקים; אבן גיר; קונגלומרט.

UMBER-אומבה

LIMESTONE אבן גיר

BEDROCK סלע

ד"ר א. או. א, לנברג

230
1350
5800
4050
6000
7800
11850
7720
7820
4230
4300
3660
4490
6160

14.5
32.5
37.2
45.0
69.9

Figure 1

Correlation between resistivity diagram (relative resistivities), log of calculated resistivities (true resistivities), and bore-hole data at umber site.

Geoelectric data		Bore-hole data	
0.0— 6.0 m	resistivities below 1000 ohm-cm	0.0— 6.5 m	loam and breccie
6.0—14.0 m	medium low resistivity (1350 ohm-cm)	6.5—14.5 m	limestone breccie with loam
14.0—44.0 m	medium high resistivities between 4000—6000 ohm-cm, 5280 on an average	14.5—45.0 m	nari, breccie, limestone with flint, limestone, umber, conglomerate; upper water horizon
44.0—72.0 m	high resistivities, above 7000 ohm-cm, 8800 on an average	45.0—69.5 m	hard porous limestone; second water horizon
72.0—99.0 m	medium high resistivities around 4000 ohm-cm	69.5—69.9 m	limestone containing some clayey material

In this correlation loam is shown by low resistivities, the intermediate complex of nari, breccie, limestone, umber and conglomerate by medium high values, and the bedrock following below by high electric resistivities. The umber itself appears to be indicated by a slight electric depression, characterized by the value of 4050 below 5800 and on top of 6000 ohm-cm. The position of the umber, therefore, can be described geologically and geoelectrically as belonging to the lower portion of the intermediate complex.

If the umber would occur in the form of a pocket of small lateral extent, it would not give a direct indication in the electric log, which shows an integrated picture of
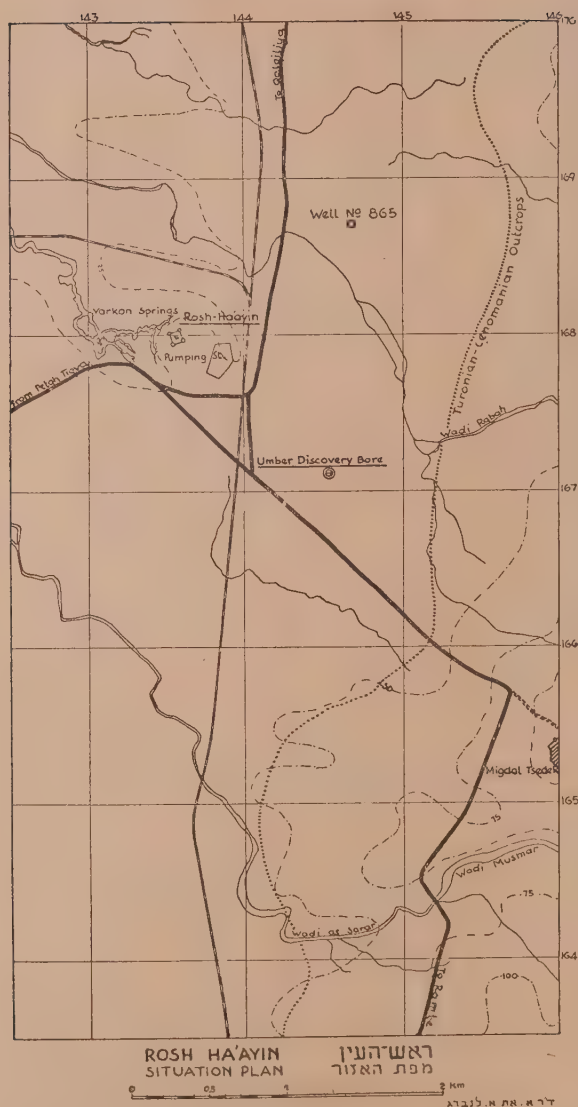
Figure 2
Yarkon spring area and umber site.

(assumed) horizontally stratified geologic and electric layers extending around the bore-hole for a distance of about 50—80 m. The correlation discussed above makes it probable, however, that the umber found at the bore site does extend laterally over some distance. This "direct" geoelectric indication together with the coincidence of the mineral-bearing formation with a definite portion of a geologic-geoelectric sequence

should make it possible to outline the conditions for the occurrence of umber in the area of Rosh Ha'Ayin. Further prospecting has been carried out, and an extension of the deposit in a northwestern, northern and eastern direction may be assumed.

GENERAL CONCLUSIONS

The origin of the umber whose main mineral constituents are earthy iron and manganese ore, is connected with the origin of the Rosh Ha'Ayin springs, situated about 1.5 km farther west-northwest at an elevation of 17 m above sea level. Blake[1] assumed these springs to rise on a NNE fault near the western edge of the Turonian shelf. Geophysical surveys in the eastern part of the Central Coastal Plain (Loehnberg and Loehnberg, 1952) have shown, however, that the springs do not lie on a main fault; neither has the continuation of the Turonian-Cenomanian block within shallow depths into the neighbourhood of the springs been confirmed by bore-hole and geophysical data.

Unconsolidated post-Cretaceous layers have been found at the umber discovery bore to 40 m depth or more, i.e. to below sea level. Pleistocene-Pliocene sand or sandstone formations have not been encountered in any of the shallow bores or wells near the spring area. Turonian bedrock may have been struck at about 8 m above sea level at Well No. 865[4]. At sites tested geophysically between the railway line and the springs, the depth of the bedrock was calculated at about 40 m below sea level.

The springs must, therefore, be assumed to rise in an "intermediate" complex, consisting of a cover of loam followed by breccie and gravel partly embedded in loam, blocks of limestone, swamp deposits (bog ore $=$ umber) and conglomerate. These layers are the result of shore conditions and of sheet and fluviatile erosion acting on an old Turonian-Cenomanian landsurface in the corner of a bay near the mouth of a river bed east of Rosh Ha'Ayin. Thus, spring conditions are seen to have existed also further east of the present spring area, most probably connected with the mouth of the ancient Wadi Rabah (Figure 2), and possibly also Wadi Musmar-Sarida. Both these wadis, especially the latter, cut deep back into the Judean mountains.

Whereas the present position of the source of the Yarkon at Rosh Ha'Ayin is of relatively recent geological times, the wider area near the springs, especially in the east-southeast direction, indicates ancient delta and debouchement conditions; a conclusion based on geological and hydrographical data and confirmed by mineralogical and geophysical evidence pointing to the occurrence of terra umbra as a swamp deposit.

The above work was carried out during World War II for the technical services of the British Army and British Air H.Q., Levant, in search for a bore-hole to supply water to the R.A.F. camp at Rosh Ha'Ayin. In subsequent years, additional investigations have been made. Drilling was carried out by Hassadeh, Ltd., Tel Aviv.

REFERENCES

1. BLAKE, G. S., 1939, Geological Map of Palestine.
2. LOEHNBERG, A. and LOEHNBERG, E., 1952, *Israel Exploration Jour.*, 2.
3. LOEHNBERG, A. and LOEWENSTEIN, A., 1936, *Beitr. z. angew. Geophy.*, 6, 67.
4. Water Register, compiled by the Mandatory Government.

LETTERS TO THE EDITOR

On some MACHILIDAE and LEPISMATIDAE from Israel, Cyprus and Arabia (Thysanura, Insecta)

This paper constitutes a report on some *Thysanura* received through the courtesy of Dr. J. Wahrman, Hebrew University, Jerusalem, Israel, and Mr. G. A. Mavromoustakis, Limassol, Cyprus as well as the locality records of some Arabian specimens received for determination from Dr. Theresa Clay, British Museum (Nat. Hist.).

We are now able to describe a new and very conspicuous lepismatid, *Ctenolepisma wahrmani*, from Israel, as well as to add new locality records for several species formerly known from this country. The genus *Lepismachilis* (with an undetermined species) as well as the lepismatids *Ctenolepisma ciliata* (Dufour), *Ctenopisma lineata pilifera* (Lucas) and *Lepismina persica* Escherich, are new for Israel.

As for the Island of Cyprus, we are not aware of any previous citation of *Thysanura*, with the exception of *Thermobia aegyptiaca* (Lucas)[1]. We are now able to present a comparatively large list of Cyprian *Thysanura*:

MACHILIDAE: *Lepismachilis* spec., *Machilinus rupestris* (Lucas).

LEPISMATIDAE: *Lepisma saccharina* L., *Lepisma wasmanni* Moniez, *Allacrotelsa kraepelini* (Escherich), *Thermobia aegyptiaca* (Lucas), *Thermobia domestica* (Packard), *Ctenolepisma lineata pilifera* (Lucas), *Ctenolepisma longicaudata* Escherich, *Ctenolepisma michaelseni* Escherich, *Ctenolepisma targionii* (Grassi and Rovelli).

The species listed above are typically Mediterranean, a few having an almost world-wide distribution. No endemisms have been found as yet. Additional species may be expected, such as several machilids, one or the other *Lepisma* and *Ctenolepisma*, and perhaps some lepismatids of the subfamily NICOLETIINAE.

The Arabian species, few in number, all belong to common species.

FAMILY MACHILIDAE

Lepismachilis spec.

Material examined: CYPRUS: Troodhitissa, 8.VIII. 1950, J. Wahrman col. (1 male, Hebrew University, TH 28). ISRAEL: Aqua Bella, 10.V.1950, J. Wahrman col. (2 females, Hebrew University, TH 6).

This species is very similar to *Lepismachilis y-signata* Kratochvil, 1945, described from Czechoslovakia, and *Lepismachilis handschini* Wygodzinsky, 1951, from Turkey. It agrees with both species in general morphological characters and the eye-pattern (the latter being unknown for *handschini*). As we have never seen actual specimens of *y-signata*, the original description being rather short, and considering the lack of clear-cut specific characters in most species of this genus, we prefer not to describe the species, which is probably new.

Machilinus kleinenbergi (Giardina, 1900)

Material examined: YEMEN: Top of Yebel Kohl, ca. 9500 ft., 1.II.1936, Dr. H. Scott col. (2 males, Brit. Mus. 1938—246).

Machilinus rupestris (Lucas, 1846)

Material examined: CYPRUS: Kannoures Spring and Valley, 7.VIII.1950, Steinitz col. (3 females, Hebrew University); Kannoures, 7.VIII.1950, J. Wahrman col. (6 females, 3 males, Hebrew University TH 35); Spring (Vrisi) of Kykko, 8.VIII. 1950, H. Steinitz col. (1 male, Hebrew University TH 43); Livadhiou, 9.VIII.1950, J. Wahrman col. (1 male, Hebrew University TH 32); Cedar Valley, Livadhiou, H. Steinitz col. (1 male, Hebrew University TH 45).

FAMILY LEPISMATIDAE

Acrotelsa collaris (F., 1793)

Material examined: ISRAEL: Wadi Nafkh, opposite J. Tiwal en-Nafkh, Negev, 27.XI.1949, J. Wahrman col. (1 male, Hebrew University TH 14); Jerusalem, 6.V.1950, 7.V.1950, 18.V.1950, Wahrman col. (1 female, two males, Hebrew University TH 4, TH 5, TH 9); Jerusalem, 27.V.1950, Weiss col. (1 female, Hebrew University, TH 10). YEMEN: San'a, ca. 7900 ft., Dr. Carl Rathjens col., 6.XII. 1937 (1 female, Brit. Mus. 1938—396).

Allacrotelsa kraepelini (Escherich, 1905)

Material examined: ISRAEL: Jerusalem, 26.XII. 1949, 21.I.1950, 18.V.1950, J. Wahrman col. (1 male, 4 females, Hebrew University TH 2, TH 3, TH 9). CYPRUS: Mt. Olympus, 6.VIII.1950, H. Steinitz and J. Wahrman col. (6 females, 7 males, Hebrew University TH 33); Kannoures, 7.VIII. 1950, J. Wahrman col. (4 females, 1 male, Hebrew University TH 36); Cherkes, 5.VIII.1950, J. Wahr-

man col. (1 immature specimen, Hebrew University TH 31); Limassol, 18/19.I.1951, Mavromoustakis col. (1 female, 1 male); Yermasoyia River, 24.I.1951, Mavromoustakis col. (1 female, 1 male); Erimi, Mavromoustakis col. (1 male).

In several of the females examined the ovipositor surpasses considerably the apex of the distal spine of stylet IX.

Ctenolepisma ciliata (Dufour, 1831)

Material examined: ISRAEL: Natanya, 5.X.1950, J. Wahrman col. (1 male, Hebrew University TH 48).

Ctenolepisma lineata pilifera (Lucas, 1840)

Material examined: ISRAEL: Jerusalem, 1.VIII. 1949, J. Wahrman col. (1 female, 1 male, Hebrew University TH 24). CYPRUS: Troodhitissa, 8.VIII. 1950, J. Wahrman col. (1 · male, Hebrew University TH 27); Limassol, 24.XI.1950, Mavromoustakis col. (1 male).

Ctenolepisma longicaudata Escherich. 1905

Material examined: CYPRUS: Akrotiri, 5.VIII. 1950, J. Wahrman col. (1 female, Hebrew University TH 34).

Ctenolepisma mauritanica (Lucas, 1846)

Material examined: WESTERN ADEN PROTECTORATE: Jebel Jimif, ca. 7100 ft., 21.IX.1937, from under large stone, H. Scott col. (1 male, Brit. Mus. 1938—246); Jebel Jihaf, ca. 7000 ft., 17.IX.1937, E. B. Britton col. (1 male, 2 females, Brit. Mus. 1938—246).

The females have the inner process of the ninth coxite larger than figured by Escherich (1905). The apical joint of the labial palp of the female is slightly longer than wide, but decidedly wider than long in the male.

Ctenolepisma michaelseni Escherich, 1905

Material examined: ISRAEL: Rvivim, 30.IX.1949, J. Wahrman col. (1 male, 2 females, Hebrew University TH 13). CYPRUS: Yermasoyia River, 24.I. 1951, Mavromoustakis col. (3 · immature specimens); Limassol, 18/19.I.1951, Mavromoustakis col. (1 female, 3 males).

We accept for this species the definition as given by Stach, in various contributions.

Ctenolepisma roszkowskii Stach, 1935

Material examined: ISRAEL: Jerusalem, 8.X.1949, J. Wahrman col. (1 male, Hebrew University TH 25).

Ctenolepisma targionii (Grassi and Rovelli, 1890)

Material examined: ISRAEL: Sahl el-Hawa, Negev, 16.X.1949, 27.XI.1949, J. Wahrman col. (2 males, 2 immature specimens, Hebrew University TH 11, TH 17). CYPRUS: Limassol, 24.XI.1940, Mavromoustakis col. (9 immature specimens).

Ctenolepisma wahrmani sp.n.

Female. Maximum length (without appendages) 15.0 mm. General shape elongate, thorax distinctly wider and a little shorter than half the length of the abdomen; the latter distinctly tapering towards apex. Colour of body whitish, hypodermal pigment absent. Pattern unknown. Antennae and caudal appendages whitish, the cerci faintly annulated with violaceous. Macrochaetae of the usual type (Figure 13). Scales uniformly with very numerous longitudinal rays which do not surpass perceptibly the anterior border of the scale.

Head slightly wider than long, with the macrochaetae arranged anteriorly in regular short longitudinal rows.

Antennae not well conserved, probably not longer than body. Each joint with one basal wreath of strong bristles, apically with a few fine hairs and occasional short sensory cones.

Molar region of mandibles strongly chitinized (Figure 1).

Lacinia of the maxillae with about 7 smooth processes and several simple bristles. Palp long and slender, with short bristles, its shape as in Figure 2.

Labium broad (Figure 3), lateral lobes without bristles. Basal joint of palpus short, the second slender, with a few rather long macrochaetae, the third short, subtriangular, the apical joint strongly widened, wider than long, with 5 sensorial papillae arranged in a single file.

Lateral border of pronotum with 6—7 bristle-combs composed each of not more than 8 macrochaetae; posterior border with 1 + 1 sublateral bristle-combs of about 8 macrochaetae each. Meso- and metanotum laterally with 8—9 bristle-combs, each with 5—6 macrochaetae; posterior border with 1 + 1 sublateral bristle-combs of 6—7 macrochaetae.

Sterna of pro- and mesothorax subtriangular, of metathorax subsemicircular; all near hind border with 2 + 2 bristle-combs, and some more or less regularly arranged subapical bristle-groups. The distance between the posterior combs on pro- and mesosternum larger than, on metasternum about as large as, width of combs (Figures 4, 5).

Legs slender, elongate; their shape and chaetotaxy as in Figures 6, 7.

Abdominal tergite I with 1 + 1, tergites II—VI with 3 + 3, VII and VIII with 2 + 2 bristle combs. Infralateral combs with 9—12, lateral with 9—12 and sublateral combs with 8—10 macrochaetae. Tergite X (Figure 12) elongate, subtriangular, its point rounded, subapically with 1 + 1 bristle-combs composed of 11—13 macrochaetae.

Abdominal sternite I without, II—VI with one submedian and III—VIII with 1 + 1 sublateral bristle-combs, the median and the sublateral ones composed of about 25 machrochaetae. Stylets on last segment only. Outer processes of coxite IX (Figure 10) very short, the inner processes mode-

rately elongate, about twice as long as wide at their base, apically on disc with a few irregularly distributed macrochaetae. Ovipositor slender, elongate, surpassing stylets by about the length of the latter. Gonapophysis composed of 50—55 joints, these with a few short bristles: simply rounded apically.

Caudal appendages about as long as body.

Material examined: ISRAEL: Wadi Menaiye, Negev, 1.II.1950, J. Wahrman col. (1 female holotype, 1 female paratype, Hebrew University TH 1).

This conspicuous species which we take much pleasure in dedicating to its collector, belongs to a group characterized by the presence of $3 + 3$
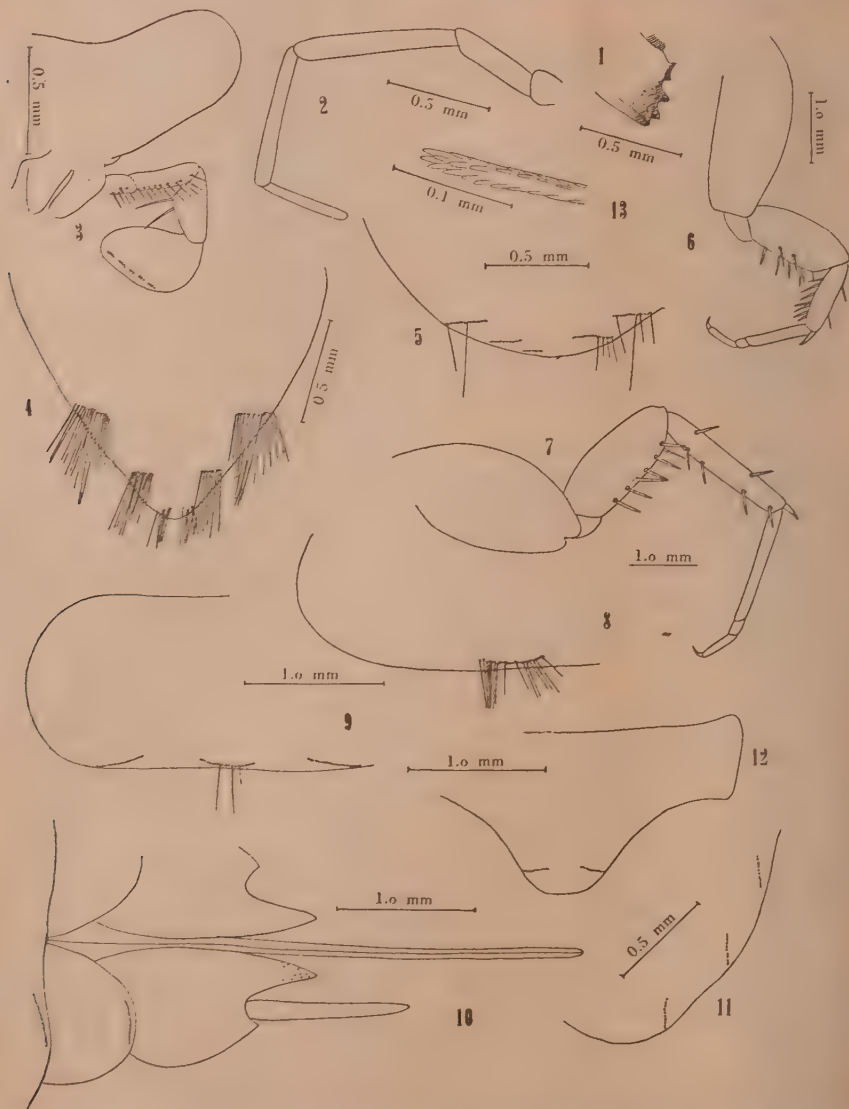
Ctenolepisma wahrmani sp.n., Female: (1) Apex of mandible. (2) Maxillary palp. (3) Labium with palp. (4) Prosternum. (5) Apical portion of metasternum. (6) Fore leg, with macro-chaetae of femur and tibia. (7) Hind leg, with macrochaetae of femur and tibia. (8) Abdominal sternite II. (9) Abdominal sternite V. (10) Genital region. (11) Postero-lateral border of abdominal tergite VI. (12) Tergite X. (13) Apex of macrochaeta of head.—Wygodzinsky del.

bristle-combs on abdominal tergites II—VI, and only one pair of stylets. Within this group, *wahrmani* approaches *michaelseni* Escherich and *roszkowskii* Stach, these three species being characterized by the presence of median bristle-combs on abdominal sternites II—VI. *Ctenolepisma wahrmani* differs from both amongst other characteristics by its much larger size and especially by the very differently shaped 10th tergite.

Lepisma saccharina L., 1758

Material examined: CYPRUS: Limassol, 18/19.I. 1951, Mavromoustakis col. (1 female).

Lepisma wasmanni Moniez, 1897

Material examined: CYPRUS: Limassol, in ants' nest, 25.X.1950, Mavromoustakis col. (numerous females and males); Erimi, 3/6.X.1950, 7.XII.1950, Mavromoustakis col. (several females and males).

Lepismina persica Escherich, 1905

Material examined: ISRAEL: Jerusalem, 20.X.1950, Zajicek col. (1 male, Hebrew University, TH 47); Naqb Gharib, Negev, 15.X.1949, J. Wahrman col. (1 immature female, 1 male, Hebrew University TH 21).

This species, which is new for Israel, differs from *L. aurisetosa*, the other species known from the country, by its relatively longer body, more than twice as long as wide, and the distal joint of the labial palpus which is not wider than long. *L. persica* is very much like *L. aurisetosa* Wahlgren, as redescribed by Stach (1935), but differs from the latter by the presence of submedian macrochaetae on the first abdominal tergite.

Lepismina plurisetosa Wygodzinsky, 1942

Material examined: ISRAEL: Haleiqim Plain, Negev 14.X.1949, J. Wahrman col. (1 male, I immature specimen, Hebrew University TH 23).

These insects correspond very well to our original description. The distal joint of the labial palp of the male is even slightly wider than figured by us.

Thermobia aegyptiaca (Lucas, 1842)

Material examined: ISRAEL: Wadi Lussan, Negev, 3.XII.1949, Wahrman col. (numerous females and males, Hebrew University); Naqb Gharib, Negev, 15.X.1949, J. Wahrman col. (1 female, Hebrew University TH 22); Jebel 'Ureif, Negev, 4.XII. 1949, J. Wahrman col. (2 females, Hebrew University TH 15); Mosad Rupin, 6.X.1950, J. Wahrman col. (6 males, Hebrew University TH 49); Aqua Bella, 10.V.1950, J. Wahrman col. (2 females, 1 male, Hebrew University TH 8). CYPRUS: Limassol, 24.XI.1950, Mavromoustakis col. (1 female).

Thermobia domestica (Packard, 1873)

Material examined: ISRAEL: no locality given, Wahrman col. (1 female, Hebrew University TH 26). CYPRUS: Aiya Irini, 14.VIII.1950, J. Wahr-

man col. (1 male, Hebrew University TH 38); near Kouklia, 20.VIII.1950, J. Wahrman col. (1 female, Hebrew University TH 39); Limassol, 26.X.1950, Mavromoustakis col. (2 females); Erimi, 3/6.X.1950, Mavromoustakis col. (1 male).

P. WYGODZINSKY
Instituto de Medicina Regional,
Tucuman, Argentina.

REFERENCES

1. WYGODZINSKY, P., 1942, *Rev. Brasil. Biol.*, Rio de Janeiro, 2(1).
2. ESCHERICH, K., 1905, *Zoologica*, Stuttgart, **43**.
3. STACH, J., 1935, *Ann. Mus. Zool. Pol.*, 9(4).

The Mechanism of Dissolution of Salt Crystals

The dissolution of salt in standing water has been shown to take place in such a way that only horizontal or near horizontal surfaces are attacked, whereas the others are protected by a film of saturated salt solution running down their slopes.

FIG 1a   FIG 1b

FIG 2a   FIG 2b

FIG 3
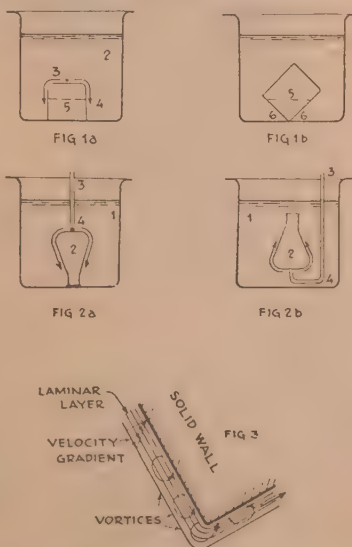
Figure 1a illustrates how a NaCl-crystal starts to dissolve in H_2O (2). A permanganate fragment (3) colours the path (4) of the saturated salt solution which protects the vertical faces of the NaCl-crystal. After several hours the crystal has been reduced to the height of the dotted line (5).

If the crystal was put into the water as shown in Figure 1b, it takes on the form indicated by the dotted line (5). The surprising fact is that face (6) also remains unattacked, and the protecting film of concentrated salt solution therefore sticks to the crystal against gravitation.
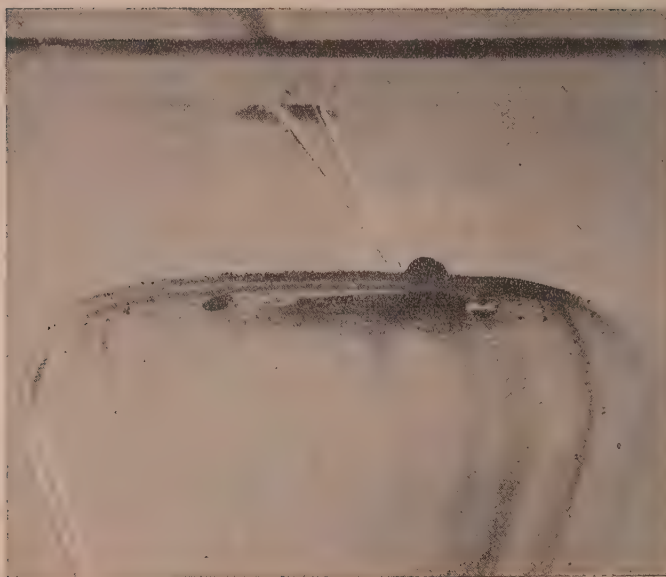
PHOTO 1   Stream of Na Cl solution

In order to study this sticking of the film more closely, the following experiment was carried out (Figure 2a)·

In a vessel filled with water, an Erlenmayer flask (2) was immersed upside down. From a cuvette (3), a concentrated salt solution was slowly run unto the bottom of the flask. A piece of permanganate (4) served to show the stream of the NaCl-solution (Photo 1).

A film of a fraction of a millimetre thickness of heavy NaCl-solution runs down the slope of (2) and sticks to it. After a few centimeters flow the film started to wiggle, to detach itself from the glass and to become turbulent. Since the sticking to the glass could possibly have been caused by surface tension, a second experiment was performed which proved that the sticking effect gas nothing to do with surface tension.

In the experiment demonstrated by Figure 2b, an Erlenmayer flask (2) was put into a concentrated NaCl solution (1). Water was run from a burette (3) through a U-tube (4) to the bottom of the flask (2); being lighter than the NaCl-solution (1), it rose again along the slope of the flask (2) sticking to its wall against gravitation. Again, after a few centimeters flow, turbulence and detachment can be observed.

Since in the first experiment the NaCl-solution seems to stick to the glass wall in preference to water and in the second experiment, water in preference to a NaCl-solution, surface tension has

to be excluded as causing the sticking effect. A similar effect can be obtained also with hot and cold water.

We explain the effect as follows:

In a viscous liquid flowing along a solid surface under the influence of an accelerating force there exists a velcocity gradient. This is connected with vortices as shown in Figure 3. These vortices make the laminar layer "stick" to the wall and it may even be observed that the water runs along the bottom of a beaker *uphill*.

In this way it can be explained that a laminar flowing film sticks to its underlying surface. If the velocity reaches a certain value, turbulence sets in, and the film is detached. This is also the case when the momentum decreases too much. Prof. S. Goldstein to whom one of us (M.R.) showed this phenomenon, remembered to have heard about it before. He also seemed to remember (but was not quite sure) that it was known under the name of Caulson-effect, but did not know of any reference. We should be obliged to readers for information on this point.

It seems that our experiments and reasonings are of some significance for understanding the dissolution and growth of crystals.

Growth as well as dissolution of crystals make the medium where it takes place inhomogeneous in specific weight.

Temperature and/or concentration of the liquid phase are changed when growth or dissolution

takes place. Some aspects of the dissolution phenomenon have already been discussed. In crystallization phenomena where up to now diffusion effects only were considered as significant, some astonishing facts (Berg[1] and Bunn's[2] experiments) might find their explanation by considering the effects of a laminar-flowing film, sticking to the growing crystal surface and passing along with or against growing dislocation.

This would explain a minimum of supersaturation in the center of a growing crystal face. The dislocation terrace grows against a film of highly supersaturated liquid slowing down to the periphery and meeting a stream the slower the further away from the center. A stream of poorer liquid then rises over the center of the dislocation.

R. BLOCH
Dead Sea Works, Ltd.,
Jerusalem.

M. REINER
Rheological Laboratory,
Hebrew Institute of Technology,
Haifa.

REFERENCES

1. BERG, N. N., *Proc. Roy. Soc.*, 1938, Á164, 79.
2. BUNN, C. W., 1949, *Crystal Growth*, p. 119, p. 152 ff. Discussions of the Faraday Society.

A Tea-Pot Effect

In connection with the problem mentioned in the preceeding letter by Bloch and Reiner[1], the following phenomenon could be observed: As most probably known to every reader, when tea is poured out of a tea-pot, the stream has the tendency not to flow in a nice ballistic curve into the cup, but to follow the underside of the spout and soil the table cloth *Every* physicist whose opinion I asked on the possible reason for this phenomenon replied that it must be due to surface tension (or, in other terminology, capillary action or adhesion). It can definitely be shown by experiments similar to those described in the letter by Bloch and Reiner[1] that this is not the case. However, while the "Caulson effect" may be operative in some cases, an effect quite distinct from the "Caulson effect" can be isolated. This is shown in the two following photographs. In the first a stream of salt-water flows *downwards* in sweet water; in the second a stream of sweet water flows *upwards* in salt water.
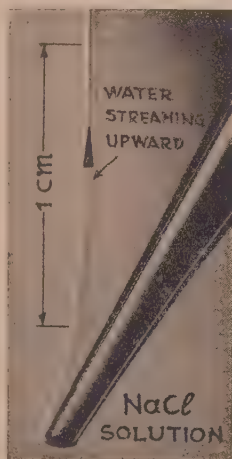
PHOTO 3
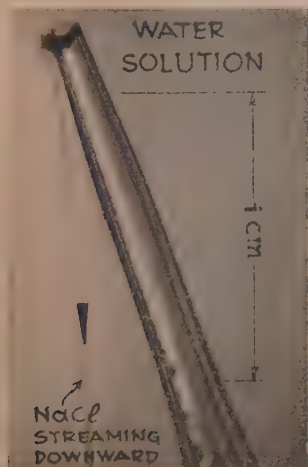
Photo Prisma

There is no *laminar layer* as in the case of the Caulson effect, nevertheless the stream does not follow a ballistic curve. On the contrary, it shows a curvature in the opposite direction. This curvature indicates *a centripetal acceleration*. However, there is no centripetal force present. The centripetal acceleration can therefore be due only to tangential tensions in the stream which have a resultant towards the centre of curvature (Figure 1).

Figure 1

It seems to me that this experiment proves that water (and probably other liquids as well) can support one dimensional stresses. This cannot be due to van der Waals forces which act isotropically in three dimensions and are the cause of the well-known isotropic strength of liquids. The present view on the internal structure of water and similar liquids is that it is a kind of polycrystal with

PHOTO 2   Photo Prisma

an intercrystalline amorphous phase, the whole difference with a solid polycrystal consisting in the temporary nature of the crystals which constantly lose and attach molecules. The tangential one-dimensional tension in the water would then be due to the water-crystals and the stream of water would behave not differently from an elastic steel chain, as shown in an experiment described by Pohl[2]. It would be worth while to perform the experiment under controlled conditions and to determine these stresses quantitatively. This may help in the establishment of a more realistic kinetic theory of liquids. I am obliged to Dr. R. Bloch for the experiments shown in the photographs. He also observed that the tea-pot effect is very small with mercury. This conforms with the fact that metal casting is not hampered by a tea pot effect.

M. REINER
*Rheological Laboratory,
Hebrew Institute of Technology,
Haifa*

REFERENCES

1. BLOCH, R. and REINER, M., 1952, *This Journal*, 2, 263.
2. POHL, *Experimental Physics*, 2.

Received August 31, 1952.

Rapid Determination of Chloride Ion by Means of the Photocolorimeter

In work with chlorine-containing insecticides, a rapid method for the determination of chloride ions was required. In the absence of the usual nephelometer, a Leitz photometer was used with success, employing the filter No. 415. Up to 120 mg Cl' per liter can thus be determined with an accuracy of 1%. As this method may be of general interest, it is briefly described here.

To a 50 cc measuring flask, were added 5 cc of the solution to be tested, 25 cc of anhydrous (or 26 cc of 96%) alcohol, 10 cc of N/10 nitric acid

and 5 cc of N/100 silver nitrate and the solution was brought up to 50 cc with distilled water. One mixes carefully, heats for 30 minutes in a water-bath of 40°, cools to room temperature, measures the absorption in the photocolorimeter (filter No. 415) and deducts the blank value (same solution, but without the silver nitrate). The result is read off the accompanying graph.

It is advisable to protect the final solution from light. The reagents should be heated before use so as to remove air bubbles.

EXAMPLES

Concentration of Cl' (mg/liter)	Galvanometer reading (average of three readings)	Extinction*	Concentration found	△
100	47.0	0.328	98.7	— 1.3
80	52.9	0.276	83.1	+ 3.1
60	64.5	0.190	57.2	— 2.8
40	73.2	0.136	41.0	+ 1.0
20	86.0	0.066	19.9	— 0.1

*) Extinction = 2 — log galvanometer reading.

A. KALUSZYNER
*Army Medical Corps,
Israeli Defense Army*

Received November 25, 1952.

Rapid Method for the Estimation of DDT in Spraying Solutions

The methods for the determination of DDT in spraying solutions are generally fairly complicated[1] in addition, they are not specified for the insecticide, but apply to all substances which lose hydrogen chloride under the conditions of the analytical method. It seemed worthwhile to study the possibility of using physical constants of the solutions, such as the specific gravity and the refractive index, for the determination of their DDT content. This is possible, of course, only if these constants are known for the solvent; in other words, the method is applicable only if the solutions are prepared locally before use.

The application of such simple methods presupposes that the solutions are ideal, i.e., that the properties of the solutions are a linear function of the composition on the whole range. The present investigation has shown that petroleum hydrocarbons do not form defined solvates with DDT, and that the specific gravity and the refractive index constitute sensitive and accurate means for determining the concentration of DDT solutions.

The experiments were carried out with DDT of m.p. 108.5° and using a pycnometer "Exax" of 25 cc capacity, mounted with a thermometer. The expansion coefficient of the petroleum solvent did not change by the dissolution of the DDT in the range studied. For the determination of the refractive index, a refractometer of Abbe type was used.

The results are summarized in Tables I—IV. From the measurements carried out at temper-

atures of 15, 20, 25, 30 and 35°, the temperature dependence of the density was calculated graphically with the following results:
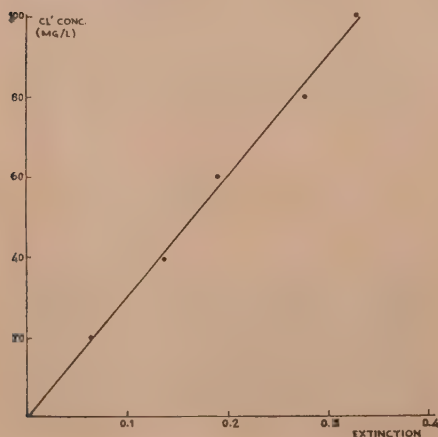
% Solutions wt/wt Solvent	Density
Solvent	$0.8044 — 78.8 \cdot 10^{-5} t$
1% Solution	$0.8085 — 81.0 \cdot 10^{-5} t$
2% Solution	$0.8125 — 82.2 \cdot 10^{-5} t$
3% Solution	$0.8152 — 77.8 \cdot 10^{-5} t$
4% Solution	$0.8188 — 77.4 \cdot 10^{-5} t$
5% Solution	$0.8223 — 77.0 \cdot 10^{-5} t$

TABLE I
Specific gravity of DDT solutions in petroleum solvent
(Weight/weight)

			15°	20°	25°	30°	35°
Solvent			0.7926	0.7888	0.7844	0.7810	0.7768
g DDT	+	g Solv.					
1	+	99	0.7964	0.7924	0.7882	0.7841	0.7803
2	+	98	0.8005	0.7960	0.7917	0.7877	0.7841
3	+	97	0.8038	0.7994	0.7956	0.7921	0.7880
4	+	96	0.8071	0.8037	0.7992	0.7956	0.7918
5	+	95	0.8108	0.8071	0.8027	0.7996	0.7953

TABLE II
Specific gravity of DDT solutions in petroleum solvent
(Weight volume)

g DDT, m·de up to 100 cc	15°	20°	25°	30°	35°
1	0.7977	0.7935	0.7893	0.7853	0.7817
2	0.8017	0.7977	0.7936	0.7900	0.7862
3	0.8060	0.8026	0.7985	0.7944	0.7907
4	0.8109	0.8072	0.8031	0.7997	0.7956
5	0.8154	0.8116	0.8077	0.8039	0.8004

TABLE III
Refractive index of DDT solutions in petroleum solvent
at 25° (Weight/weight)

	Solvent	1.4391
g DDT + g Solvent		
1	99	1.4400
2	98	1.4412
3	97	1.4421
4	96	1.4431
5	95	1.4440

TABLE IV
Refractive index of DDT solutions in petroleum solvent
at 25° (Weight volume)

g DDT made up to 100 cc	
1	1.4402
2	1.4415
3	1.4426
4	1.4440
5	1.4451

DDT, employed in actual practice, is of course not pure; however, the error introduced by using the above tables, which refer to pure DDT, is negligible.

A. KALUSZYNER
S. REUTER
Z. LEVINSON
Army Medical Corps,
Israeli Defense Army

REFERENCE

1. See e.g., 1950, Official Methods of Analysis of the A.O.A.C. 7th Ed.

A Collection of TABANIDAE (Diptera) From the Dead Sea Area of Palestine*

This is a report on a small collection of Tabanidae taken by the writer in the vicinity of Fesha Swamp which drains into the northeast side of the Dead Sea, Palestine. These observations were incidental to field work with *Phlebotomus papatasii* under the auspices of the U.S. Army Neurotropic Virus Commission during World War II. This environment was of particular interest because of its isolation and unique low altitude—some 1500 feet below sea level.

Two visits were made to the Swamp by jeep on the twenty-fifth of May and first of June 1943, and were necessarily brief because of the known presence of hostile natives in the neighbourhood. Due to the heat in the middle of the day, the flies were very active and difficult to capture. Many more were seen than were taken. Males of some species were seen resting on trunks of the low trees and shrubs, indicating that this area of fresh water seepage in an otherwise arid desert margin along the Dead Sea, was furnishing a natural and isolated breeding place for these biting flies.

The ecological gradient in this shore environment from fresh to the extreme salt water of the Dead Sea would furnish an interesting biological study of biting fly breeding under a relatively isolated situation. The following list of species taken in these environs is likely a very inadequate sample for this interesting environment, probably the lowest in altitude of anywhere on earth as far as tabanid breeding is concerned. It is of course, entirely possible that some of these strong fliers had invaded the area from distant points such as the Jordan Valley.

Chrysops
  flavipes subsp. *punctifera* Lw. 4 ♀
  buxtoni Aust. ♀
Hybomitra
  decorus (Lw.) ♀
Atylotus (*Abatylotus*)
  pulchellus (Lw.) ♂
Tabanus
  albifacies Lw. 2 ♀
  gratus Lw. 2 ♂, 9 ♀
  laetitinctus Beck. ♂, 4 ♀
  syriacus Krob. 2 ♀

The generic assignments of *H. decorus* and *A. pulchellus* are new combinations, the reasons for which have previously been discussed by the writer[1].

CORNELIUS B. PHILIP,
Rocky Mountain Laboratory,
Hamilton, Montana

REFERENCE

1. PHILIP, C. B., 1948, Bull. Soc. Fouad, 1er Ent., 32, 77.

A Vinylene Type Derivative of Glutamine

Glutamine derivatives have been found to act as inhibitors of growth of micro-organisms[1,2] and of the enzymic synthesis of glutamine by acetone-dried sheep brain preparations[3]. On the other hand, it was found that several unsaturated amino acids markedly inhibited the growth of yeast and bacteria[4,5,6]. It seems desirable, therefore, to prepare an unsaturated derivative of glutamine and to test its biological activity.

Such a derivative, N-(DL-γ-Glutamyl)-allyl-amine, can be obtained by the method previously described[1,7] for the preparation of γ-alkylamides of glutamic acid. Ten g of DL-pyrrolidonecar-boxylic acid were treated with 54 g of 33% aqueous allylamine solution for 20 days at 37° in a sealed glass tube. The filtered solution was evaporated to a syrup in a vacuum desiccator over sulphuric acid at 37°. The syrup was triturated with about 100 cc of absolute alcohol and stored in the refrigerator. The precipitate formed was filtered by suction, washed with cold absolute alcohol and dried in a vacuum desiccator over sulphuric acid. Yield, 0.4—0.6 g, m.p. 197—198° (uncorrected). For analysis, the substance was dried in vacuo over phosphorus pentoxide at about 60°. (Anal. Found: N, 15.1 (Kjeldahl); amino-N, 7.6 (Soerensen's formol titration); 7.2 (Van Slyke's ninhydrin method). Calculated for $C_8H_{14}N_2O_3$: N, 15.1; amino-N, 7.5. Halogen addition (Dam's iodine value method[8]): 33.3 mg absorbed 28.0 mg Br; calculated 28.6 mg. (Asparagine and N-(γ-glutamyl)-ethylamine did not react with Dam's reagent).

The investigation of the biological activity of this compound is under way.

RUTH ZUR
N. LICHTENSTEIN
Department of Biological and Colloidal Chemistry,
The Hebrew University, Jerusalem

REFERENCES

1. LICHTENSTEIN, N. and GROSSOWICZ, N., 1947, *J. biol., Chem.* 171, 387.
2. BACK, A., SADOVSKY, A. and LICHTENSTEIN, N., 1950, *Nature,* 166, 352.
3. LICHTENSTEIN, N., ROSS, H. E. and COHEN, P. P., *J. biol. Chem.* (in press).
4. DITTMER, K., GOERING, H.L., GOODMAN, I., and CRISTOL, S. J., 1948, *J. Amer. Chem. Soc.,* 70, 2499.
5. GOERING, H. L., CRISTOL, S. J. and DITTMER, K., 1948, *J. Amer. Chem. Soc.,* 70, 3314.
6. GERSHON, H., MEEK, J. S. and DITTMER, K., 1949, *J.Amer. Chem. Soc.,* 71, 3573.
7. LICHTENSTEIN, N., 1942, *J. Amer. Chem. Soc.,* 64, 1021
8. PLIMMER, R. H. A., 1938, *Organic and Bio-chemistry,* Sixth edition, p. 244.

Received October 9, 1952.

3,4-Epoxy-2,5-Dimethoxytetrahydrofuran: A Cyclic Acetal of Epoxysuccinaldehyde

Epoxysuccinaldehyde is of interest both as a potential starting material for the synthesis of scopolamine and because it may afford an interesting intermediate for the synthesis of a sub-stituted seven-membered cyclic ketone to be used in the synthesis of colchicine. We wish to record at this time our observations in this field because of the recent publications related to epoxysuc-cinaldehyde[1,2,3].

In view of our interest in colchicine[4], we have prepared a cyclic acetal of epoxysuccinaldehyde and have hydrolysed it in the presence of 2,4-dinitrophenylhydrazine to a product, m.p. 246—247°, whose analysis conforms with its formulation as the *bis*-2,4-dinitrophenylhydrazone of epoxysuccinaldehyde or an isomeric heterocyclic derivative.

By a procedure similar to that reported for the 2,5-diethoxy-homolog[2], we have converted 2,5-dimethoxy-2,5-dihydrofuran[5] by means of *t*-butyl hypochlorite in 90% acetic acid to 3-chloro-4-hydroxy-2,5-dimethoxytetrahydrofuran, b.p.100—103° (6 mm); yield, 75%. This preparation appears to be superior to that used by Sheehan and Bloom[3], who report b.p. 80° (0.4 mm). (Anal. Calcd. for $C_6H_{11}O_4Cl$: C, 39.5; H, 6.0; Cl, 19.4. Found: C, 39.7; H, 6.0; Cl, 19.7).

It has been our experience that the addition of *t*-butyl hypochlorite to various compounds containing olefinic bonds, in aqueous-organic solvents, yields the *trans*- chlorohydrin, as can be shown by the formation of the epoxide. For example, cyclohexene gives *trans*-2-chlorocyclo-hexanol in high yield[6]. That the *trans*- chloro-hydrin was formed also in the present case could be shown by conversion to the corresponding epoxide. albeit in poor yield.

Treatment of 3-chloro-4-hydroxy-2,5-dimetho-xytrahydrofuran in boiling xylene with dry freshly precipitated silver oxide yielded 3,4-epoxy-2,5-di-methoxytetrahydrofuran, b.p. 55—58° (2 mm); yield, 55%. After standing in the refrigerator for two weeks, the product deposited colourless needles which after filtration and washing with ice-cold petroleum ether had m.p. 39—41°. Sheehan and Bloom[3] report m.p. 43—45° for the crystalline product which they obtained in superior yield. The yield of crystalline epoxide in our hands was 10—15%. (Anal. Calcd. for $C_6H_{10}O_4$: C, 49.3; H, 6.9. Found: C, 49.1: H, 6.9). The mother liquor upon refractionation showed similar analytical data.

When 3,4-epoxy-2,5-dimethoxytetrahydrofuran was treated with a solution of 2,4-dinitrophenyl-hydrazine in ethanol containing a few drops of concentrated hydrochloric acid, crystalline material deposited after several days standing at room temperature. The material was taken up in chloro-form and chromatographed on alumina. Four bands were present in the column, one of which corresponded to 2,4-dinitrophenylhydrazine and another to a crystalline product. m.p. 245—247° (ethanol-ethyl acetate). (Anal. Calcd. for $C_{16}H_{12}O_9N_8$: C, 41.7; H, 2.6; N, 24.3. Found: C, 42.0; H, 2.8; N, 24.1). The amorphous products eluted from the other two bands in the column have as yet not been characterized.

This work has been carried out partly at the Weizmann Institute of Science and continued at Harvard University during the tenure of a National Cancer Institute postdoctoral fellowship. Further details of this investigation will be reported elsewhere.

DAVID GINSBURG
Converse Memorial Laboratory,
Harvard University,
Cambridge 38, Mass.

REFERENCES

1. HUFFORD, D. L., TARBELL D. S. and KOSZALKA, T. R., 1952, *J. Amer. Chem. Soc.*, 74, 3014.
2. STOLL, A., BECKER, B. and JUCKER, E., 1952, *Helv. Chim. Acta*, 35, 1263.
3. SHEEHAN J. C. and BLOOM, B. M., 1952, *J. Amer. Chem. Soc.*, 74. 3825.
4. GINSBURG, D. and PAPPO, R. (in press).
5. CLAUSON-KAAS, N., LIMBORG, F. and FAKSTORP, J., 1948, *Acta Chem. Scand.*, 2, 109. We are grateful to Dr. Niels Clauson-Kaas for a generous supply of this compound.
6. GINSBURG, D., unpublished results.

The Action of *t*-Butyl Hypochlorite on Cholesterol and Cholestan-3-one

When cholesterol is dissolved in *t*-butanol and the refluxing solution is treated with an excess (2—3 moles) of *t*-butyl hypochlorite, both chlorination and oxidation of the secondary hydroxyl function to a carbonyl group occur. The intermediate chlorine-containing product was not isolated but could be dehydrochlorinated by treatment with dimethylaniline or with 2,4-dinitrophenylhydrazine. With the latter reagent, direct isolation of the 2,4-dinitrophenylhydrazone of cholesta-4,6-dien-3-one is possible in 60—65 % yield. This compound forms deep red needles, m.p. 230—231° (alcohol-ethyl acetate). $\lambda\lambda_{max}$ 309, 403m μ; log $\varepsilon\varepsilon_{max}$ 4.2, 4.5. The *UV* absorption curve is virtually superimposable on the curve reported for this compound by Djerassi and Ryan[1]. Djerassi[2] reports m.p. 227—229° for this compound[3].

Chlorination of cholestan-3-one in acetic acid solution with one mole of *t*-butyl hypochlorite affords 2β-chlorocholestan-3-one in 76 % yield. The chloro-ketone melts at 178° (acetic acid). $\alpha_D^{24} = +46.2°$ (CHCl$_3$) (c = 1.83). (*Anal.* Calcd. for C$_{27}$H$_{45}$OCl: C, 76.9; H, 10.7; Cl, 8.4. Found: C, 77.1; H, 10.5; Cl, 8.4). The position of the halogen is apparent from the fact that treatment of the chloro-ketone with 2,4-dinitrophenylhydrazine yields the 2,4-dinitrophenylhydrazone of cholest-1-en-3-one, m.p. 219—220°, which showed no depression on admixture with an authentic specimen. Djerassi[2] reports m.p. 218—220° for this compound.

The configuration of the chlorine atom at C-2 is apparent from the following evidence: Sodium borohydride reduction of the chloro-ketone in ethanol solution gave an 84% yield of the crystalline chlorohydrin, 2β-chlorocholestan-3β-ol, m.p.

105—106° (ethanol), $\alpha_D^{24} = +17.8°$ (CHCl$_3$) (c = 2.20). (*Anal.* Calcd. for C$_{27}$H$_{47}$OCl: C, 76.6; H,11.1; Cl, 8.4. Found: C, 76.4; H, 11.0: Cl, 8.3). This chlorohydrin gives a precipitate with digitonin and upon refluxing with methanolic potassium hydroxide gives a ketone identical with cholestan-3-one in 81 % yield but does not give an epoxide[4]. Thus, the hydroxyl at C-3 is present in the β-orientation and the chlorine at C-2 which is *cis*- with respect to the hydroxyl at C-3 must also be present in the β-orientation. It is not surprising that the 3β-hydroxy-compound was isolated (the 3α-hydroxy-isomer could not be isolated) as in the former the reduction product has assumed the more stable equatorial conformation. In the unisolated isomer, the 3α-hydroxyl would be polar.

Experiments are being continued on the application of *t*-butyl hypochlorite as a halogenating agent in the steroid series, and the results will be reported elsewhere. We wish to acknowledge gratefully the benefit of stimulating discussion with Prof. L. F. Fieser and his interest in this work.

DAVID GINSBURG
Converse Memorial Laboratory,
Harvard University,
Cambridge 38, Mass.

REFERENCES

1. DJERASSI, C. and RYAN, E., 1949, *J. Amer. Chem. Soc.*, 71, 1000.
2. DJERASSI, C., *ibid.*, 1003.
3. We wish to thank Dr. A. L. WILDS for a specimen o cholesta-4,6-dien-3-one.
4. Compare BARTLETT, P. D., *J. Amer. Chem. Soc.*, 1935, 57, 224.

Two New Species of Foraminifera from Israel

In an earlier publication[1] reference has been made to two new species of smaller foraminifers, occurring in our country: *Gyroidinoides* nov.sp. from the Santonian-Campanian, and *Pseudovalvulineria* nov.sp. from the Danian-Paleocene.

Both species are herein described and figured.

Gyroidinoides Pseudosimiensis Reiss, nov. sp.
1952 *Gyroidinoides* nov.sp. Reiss[1], p. 40.

Holotype: Figure 1, 50 ×, from the upper part of the Santonian-Campanian of Har Tuv (Israel), Sta. 22a (type-locality). Deposited in the collec-
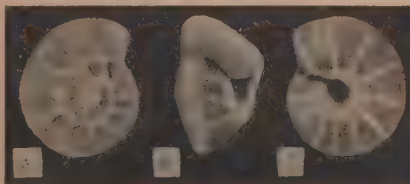

Figure 1
Gyroidinoides pseudosimiensis Reiss Holotype. 50×.

tion of the Geological Institute of Israel, Jerusalem (No. 524).

Description: Test trochospiral, nearly planoconvex, spiral side flat or very slightly convex, umbilical side strongly convex; peripheral margin sharply rounded; 2.5 or 3 whorls visible on the spiral side, only the last one on the umbilical side; 10—12 chambers in the last whorl, usually 11; chamber-sutures almost straight and slightly oblique, broadly limbate and raised above the surface on the spiral side, merging into the limbate and raised spiral suture; sutures on the umbilical side limbate, slightly raised above the surface, merging into a thickening, which surrounds the deep and fairly large umbilicus; septal aperture interio-marginal, extending into an umbilical aperture, covered by umbilical lips; chamber-walls finely perforate; diameter: 0.35—0.50 mm, height: 0.25—0.30 mm.

Remarks: This species resembles *Gyroidinoides simiensis* Cushman and McMasters 1936 (hence the *derivatio nominis*), described from the Middle Eocene of California, but differs from it in its sharper perpheral margin, in the lack of the raised knobs of the sutures near the umbilicus and in the profile of the umbilical side, which is nearly straight in the present species, but convex in *G.simiensis*.

Occurrence: Gyroidinoides pseudosimiensis is an excellent guide-fossil for the upper part of the Santonian-Campanian of Israel, where it occurs frequently to abundantly. Rare specimens have been found in the Lowest Maestrichtian.

Pseudovalvulineria avnimelechi Reiss, nov.sp.
1952 *Pseudovalvulineria* nov.sp. Reiss[1], p. 43

Holotype: Figure 2, 50×, from the lower part of the Danian-Paleocene of Tzor'a (Israel), Sta. 12 (type-locality). Deposited in the collection of the Geological Institute of Israel, Jerusalem (No. 459).
. *Description:* Test trochospiral, nearly planoconvex, spiral side flat or slightly convex in the middle part, umbilical side strongly convex; peripheral margin distinctly keeled, the rounded keel not always reaching the last 1—2 chambers; 9—12 chambers in the last whorl, generally 10; 3 or 3.5 whorls visible on the spiral side, only the last one on the umbilical side; earlier whorls on the spiral side usually obscured by a cover of clear shellmaterial; spiral suture limbate and usually raised

above the surface, merging into the peripheral keel; sutures between the chambers on the spiral side flush with the surface and appearing as dark, fine lines, oblique and slightly curved, more distinct in the last whorl and in the latter portion of the preceeding one; on the umbilical side the sutures are limbate and raised above the surface, radial and curved, sometimes slightly depressed in the last 1—2 chambers; irregular depressions run along the sutures on the umbilical side, radiating from the umbilicus; umbilicus small, for the greatest part covered by umbilical lips; aperture interio-marginal, continuing into a short umbilical aperture, covered by the lips of the last chambers; chamber-walls perforate, coarsely so on the umbilical side. Diameter: 0.4 — 0.55 mm. Height: 0.25 — 0.35 mm.

Remarks: Pseudovalvulineria avnimelechi is apparently closely related to *Pseudovalvulineria gracilis* (Marsson) Brotzen, described from the Chalk of Ruegen, and occurring in the Maestrichtian of the Baltic regions, of Scandinavia and of Israel. Specimens of *P.gracilis* from the Moeenian of Sweden and Denmark, kindly supplied by Dr. F. Brotzen, agree very closely with specimens of this species from the Maestrichtian of Israel.

P.gracilis differs from *P.avnimelechi* by its generally flatter and distinctly biconvex form, its broader and sharper peripheral keel, as well as by its less broadly limbate and less raised sutures on the umbilical side.

Specimens showing transitional characters between *P.gracilis* and *P.avnimelechi* occur in the deepest layers of the Danian-Paleocene of Israel.

This species has been named in honour of Dr. M. Avnimelech, of the Hebrew University, Jerusalem, Head of the Department of Paleontology, Geological Institute of Israel.

Occurrence: This species is an excellent guidefossil for the whole Danian-Paleocene of Israel, where it occurs frequently.

Z. REISS
Laboratory of Micropaleontology,
Geological Institute of Israel

REFERENCE

1. REISS, Z., 1952, *Bull. Res. Counc. of Israel*, 2, 27.

Figure 2
Pseudovalvulineria avnimelechi Reiss. Holotype. 50×.

On the Occurrence of Globotruncana calcarata Cushman 1927 in the Upper Cretaceous of Israel

Rare specimens of *Globotruncana calcarata* Cushman[1] have been found in a drill-sample from a boring near Tulkarem, on the Jerusalem-Haifa railway.

This is the first record for this species from the eastern Mediterranean region and as such of particular interest.

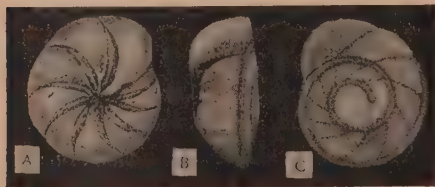The boring is 80 m deep. The section is as follows:

0—10.60 m.	Soil and gravels	Quaternary
—57.80 m.	Somewhat detritic, slightly limonitic and phosphatic chalk	Campanian Santonian-
—66.50 m.	Bituminous, calcareous marl	
—80.00 m.	Limestone (so-called "Mitzi hilu")	Turonian

Five specimens of *Globotruncana calcarata* Cushman have been found in the sample taken at 25.60 m depth (sample No. 2138—75 PG, Collection Geol. Institute of Israel). The rock-sample is a somewhat detritic, slightly limonitic and phosphatic chalk, very rich in species and individuals of smaller foraminifera, also fairly rich in *Ostracoda*, fish-teeth and coprolithes. Benthonic foraminifera are numerically predominant, but planktonic ones are also fairly frequent.

The foraminiferal assemblage contains among others:

Spiroplectammina laevis (Roemer), var. *cretosa*
   Cushman
Dorothia glabrella Cushman
Palmula suturalis Cushman
Palmula rugosa (d'Orb.) s.l.
Buliminella carseyae Plummer
Bulimina prolixa Cushman and Parker
Bulimina reussi Morrow
Bulimina kickapooensis Cole
Loxostomum gemmum (Cushman)
Bolivinoides decorata (Jones)
Allomorphina cf. *A. whangaia* Finlay
Valvulineria cf. *V. lenticula* (Reuss)
Gyroidinoides nitida (Reuss)
Gyroidinoides sp. (= *Gyroidinoides* nov. sp. Reiss
   1952[2])*
Osangularia cordieriana (d'Orb.)
Cibicides semicomplanata (Cushman and Hedberg)
Cibicides cf. *C.abudurbensis* Nakkady
Cibicides beaumontiana (d'Orb.)
Globigerinella aspera (Ehrenberg)
Globigerina cretacea d'Orb.
Globotruncana arca (Cushman)
Globotruncana cretacea Cushman
Globotruncana rosetta (Carsey)
Globotruncana lapparenti lapparenti Bolli
Globotruncana fornicata Plummer

*) The description of this species is given elsewhere in this Bulletin (p. 269).

Globotruncana cf. *G.globigerinoides* Brotzen
Globorotalites sp. (= *Globorotalites* sp. Reiss
   1952[2]), small form.
Gümbelina striata (Ehrenberg)
Gümbelina globulosa (Ehrenberg)
Gümbelina plummerae Loetterle
Nonionella sp.
Various species of *Nodosaria, Dentalina, Lenticulina, Marginulina, Frondicularia*, etc.

This assemblage points to a Campanian—particulary Upper Campanian—age (see Reiss 1952[2]). In complete sections it is followed by typical maestrichtian fauna, containing *Palmula reticulata, Bolivinoides draco, Bolivina incrassata, Stensiöina pommerana, Globotruncana stuarti, Pseudotextularia elegans*, etc.

Globotruncana calcarata has been recorded hitherto from the Americo-Carribbean area, from Europe, North Africa and the East Indies. The records are as follows:

Americo-Caribbean area:
Taylor formation and lower Navarro formation (Neylandville marl) of Texas; particularly abundant in the Pegan Gap Chalk and Wolfe City Sand members of the Taylor formation[3].

Upper Papagallos formation (below the top) of the Tampico region, Mexico[4] (see, however, stratigraphical distribution of *G.calcarata* fide Marie[5]: top Papagallos-basal Mendez.(?)).

Reworked specimens of *Globotruncana* cf. *G. calcarata* occur in the Upper Eocene of Trinidad[6].

Europe:
Campanian of the Aquitaine basin[5,7,8,9,10] and of Spain[5].

Upper Senonian of the Gamsa basin (Austria) (Maestrichtian according to Wicher[11], Campanian according to Bartenstein[12]).

Senonian of Rumania (Maestrichtian according to Wicher[11], Campanian according to Bartenstein[12]).

Upper Campanian of Romny, Ukraina[12].*

North Africa:
Campanian of Morocco, Algeria and Tunisia[5,7,8,13].

* During the printing of the present note, A. Orlini's publication on *G. calcarata* (1949, *Riv. Ital. Paleont. Strat.*, 55 No. 1) has come to hand. Orlini records *G. calcarata* from the Santonian-Campanian of Italy.
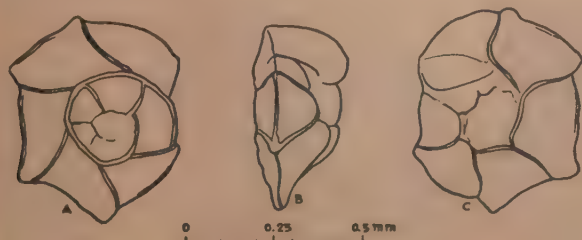

Figure 1
Globotruncana calcarata Cushman.
Campanian. Israel.

0   0.25   0.5 mm

East Indies:
Maestrichtian of Ceram[14].

The stratigraphical distribution of this characteristic species has been repeatedly discussed by various authors (see references). Wicher[11] considered this problem in much detail. He speaks about a "*calcarata*-level" of the Maestrichtian and arrives at the conclusion that the *G.calcarata*-bearing strata of America and Europe must be placed in the Lower Maestrichtian and, therefore, the Upper Papagallos of Mexico must be correlated with the Lower Maestrichtian of Europe and not with the Upper Campanian, as advocated by Keller[15]. Bartenstein[12], on the other hand, regards *Globotruncana calcarata* as characteristic for the Campanian and places in this substage the strata with *G.calcarata* regarded by Wicher[11] as being of Maestrichtian age. Indeed, Wicher's evidence of the Maestrichtian age of the *calcarata*-bearing strata of Mexico and Europe is not fully convincing and their Campanian age, in particular Upper Campanian, seems more likely. Marie[5],[8] states that the *G.calcarata*-bearing horizon in North Africa is only 1 m (!) thick and ought to be correlated with the *calcarata*-bearing strata of Mexico and Spain, which are of Campanian age. He regards, however, these strata as being of Lower Campanian age (in an earlier publication he correlates them with the Upper Santonian). This view has been rejected by other authors (see discussion Marie[9]), who regard these strata as being of Upper Campanian age. Glaessner[13] also arrives at the conclusion that the *G.calcarata*-bearing strata of North Africa are of Campanian age. It is, however, noteworthy that, according to his table this species occurs in the whole Campanian. Kikoine[10] states that in the North-Pyrennean region *Globotruncana calcarata* is apparently restricted to the Campanian.

From this account it appears, however, that a correlation of all *calcarata*-bearing strata in the Tethys-area either with the Upper Campanian or the Lower Maestrichtian is unjustified: although *G.calcarata* occurs mainly in strata of Campanian age—particularly Upper Campanian—it has also been recorded from strata of Maestrichtian age, such as the *Palmula reticulata*-bearing Upper Cretaceous of the island of Ceram, or the Navarro formation of Texas, which is usually correlated with the Maestrichtian of Europe.

On the basis of these evidences we arrive at the conclusion that *Globotruncana calcarata* occurs in the Campanian, as well as in the Maestrichtian of both hemispheres, although it is most frequently found in strata of Upper Campanian age.

Z. REISS
Laboratory of Micropaleontology,
Geological Institute of Israel.

REFERENCES

1. CUSHMAN, J. A., 1927, *Contrib. Cushman Labor. Foram. Research*, 3.
2. REISS, Z., 1952, *Bull. Res. Counc. of Israel*, 2, 37.
3. CUSHMAN, J. A., 1946, U. S. *Geol. Survey Prof. Paper*, 206.
4. WHITE, M. P., 1928-1929, *Jour. Paleontology*, 2, 3.
5. MARIE, P., 1941, *Mem. Mus. Nat. d'Hist. Nat.*, 12, fasc. 1.
6. BOLLI, H., 1951, *Jour. Paleontology*, 25, pt. 1.
7. MARIE, P., 1936, *C. R. S. Acad. Sci.*
8. MARIE, P., 1943, *C. R. S. Soc. Geol. France.*
9. MARIE, P., 1947, *C. R. S. Soc. Geol. France.*
10. KIKOINE, J., 1947, *C. R. S. Soc. Geol. France.*
11. WICHER, C. A., 1949, *Bull. Mus. d'Hist. Nat. Pays Serbe*, Ser. A-2.
12. BARTENSTEIN, H., 1948, *C. R. S. Soc. Geol. France.*
13. GLAESSNER, M. F., 1937, *Moscow Univ. Public.* ("Problems of Paleontology") 1, fasc. 1.
14. VAN DER SLUIS, J. P., 1950, *Public. Min. Geol. Institut. Rijks Univ. Utrecht.*
15. KELLER, M., 1939, *Abh. Erdölgeol. Institut. Ser. A*, 116, Moskau.

The Dielectric Constant of Aqueous Thixotropic Gels at Microwave Frequencies

A number of theories have been advanced to explain the reversible sol-gel transformation called thixotropy[1],[2]. One theory[3],[4] assumes that the colloidal particles are surrounded by layers of bound solvent called 'lyospheres'. The rigidity of these gels is attributed largely to these layers. The thickness of these layers is assumed to be as large as 100 molecular diameters. When shearing, these layers are broken up, and the gel becomes liquid. In aqueous gels the water layers are presumably bound mainly by electrical forces.

It is well known that polar liquids show dielectric dispersion at microwave frequencies[5]. Water, for example, has a maximum dielectric loss, $\tan \Theta$, in the cm region[6],[7]. Water bound to crystals, as in certain hydrates, has not such a characteristic loss at these frequencies. For example at 3 cm, the loss factor for powdered $CuSO_4.5H_2O$ at room temperature is approximately 0.002[8],[9], whereas that of water under same conditions is 0.55.

According to the lyophilic theory part of the water at least should be bound irrotationally to the colloidal particles. One would expect, therefore, a change of dielectric constant $\acute{e}$ and dielectric loss factor $\tan \Theta$ during a sol-gel transformation. We have investigated the dieletric constant and loss of alumina molybdate gels[10] at 3.1 cm. We have found no change during the sol-gel transformation. In both states, $\acute{e}$ and $\tan \Theta$ had a value close to that of distilled water.

The lyophilic theory has been criticized in the past by a number of authors[11],[12]. Our results can be taken as a further proof that there are no changes of oriented bound layers of solvent in thixotropic gel transformations.

The 'random mesh' or 'scaffolding' theory[2] assumes that the colloidal particles alone form a structure extending through the whole gel. Molecular binding between the colloidal particles accounts for the rigidity of the gels. According to such a theory also one would expect marked changes of dielectric constant and loss factor during a sol-gel transformation. Our results, how-

ever, cannot be interpreted as a refutation of this theory. The very large dielectric constant of water may well mask any smaller changes due to the colloidal particles. The colloidal particles moreover would show dielectric relaxation on effects only in the Mc range, We hope to extend our measurements to polar thixotropic gels in non-polar organic media.

The measurements were made by means of a standing wave meter according to the Roberst-Von Hippel method[13],[14] as well as in a liquid cell whose length was variable[15]. The authors wish to thank Mr. Loewensohn of the machine shop of the Department of Physics for constructing a large part of the microwave equipment.

W. LOW
J. BARUCH
Department of Physics,
The Hebrew University, Jerusalem

REFERENCES

1. ALEXANDER, A. E. and JOHNSON, P., 1949, *Colloid Science*, Oxford Univ. Press.
2. GOODEVE, C. F., 1939, *Trans. Faraday Soc.*, 35, 343.
3. HAUSER, E. A., 1931, *J. Rheology*, 2, 5.
4. See ref. (1), page 124.
5. DEBYE, P., 1945, *Polar Molecules*, Dover, Publ. N.Y.
6. COLLIE, C. H., HASTED, J. B. and RITSON, D. M., 1948, *Proc. Phys. Soc.*, 60, 145.
7. LANE, J. A. and SAXTON, J. A., 1952, *Proc. Roy. Soc.* A213, 400.
8. BARUCH, J. and LOW, W., to be published.
9. FREYMANN, M. and FREYMANN, R., 1951, *Comptes Rendus*, 232, 401.
10. Preparation of these gels can be found in a paper by PRASAD, M. et al., 1952, *J. Coll. Sci.*, 7, 178.
11. USHER, F. L., 1929, *Proc. Roy. Soc.*, A125, 143.
12. RUSSELL, J. L. and RIDEAL, E. K., 1936, *Proc. Roy. Soc.*, 154, 540.
13. ROBERTS, S. and VON HIPPEL A., 1946, *J. App. Phys.*, 17, 610.
14. DAKIN, D. W. and WORKS C. N., 1947, *J. App. Phys.*, 18, 789.
15. CROUCH, G. E., 1948, *J. Chem. Phys.*, 16, 364.

A New Synthesis of Cyclohexenones

When the enol-ethers of cyclohexane-1, 3-diones are reduced with lithium aluminum hydride and the reaction products decomposed under acidic conditions, cyclohexenones are obtained. As a number of recent papers describe essentially the same method[1],[2],[3], our results are communicated here.

Cyclohexane-1, 3-dione is converted into the methyl ether of its enol form by diazomethane: b.p. 73° at 0.5 mm; m.p. 37°. (*Anal.* Calcd. for $C_7H_{10}O_2$: C, 66.6; H, 8.0; Found: C, 66.8; H, 8.1). Reduction with lithium aluminum hydride[4] and acidification afforded a good yield of $\triangle^4$-cyclo-hexenone (I), b.p. 70° at 26 mm; λ_{max} 225 mμ; $E = 11,270$. The high extinction coefficient compares very favourably with the best value ($E = 10,250$) obtained by one of us using a different method for the preparation of (I)[5].

Using the same procedure, 2-methyl-cyclohexane-1,3-dione was converted into the enol-methyl ether, b.p. 90° at 0.8 mm, m.p. 46°. (*Anal.* Calcd. for $C_8H_{12}O_2$: C, 68.6; H, 8.6; Found: C, 68.3; H, 8.8), and further into 2-methyl-cyclohex-2-enone (II), b.p. 74—75° at 25 mm. (*Anal.* Calcd. for $C_7H_{10}O$: C, 76.3; H, 9.2; Found: C, 76.4; H, 9.5). λ_{max} 234.5 mμ; $E = 9850°$.

2-Phenyl-cyclohexane-1,3 dione was synthesized as follows: benzyl cyanide was condensed with diethyl glutarate in the presence of sodium methoxide, to give ethyl 5-keto-6-cyano-6-phenyl-caproate (III) which crystallizes as monohydrate, m.p. 65° (55°). (*Anal.* Calcd. for $C_{15}H_{17}NO_3H_2O$: C, 65.0; H, 6.8. Found: C, 64.9; H, 6.9). Hydrolysis, decarboxylation and reesterification afforded ethyl 5-keto-6-phenyl-caproate (IV), m.p. 43—45° (*Anal.* Calcd. for $C_{14}H_{18}O_3$: C, 71.8; H, 7.7; Found: C, 72.2; H, 8.0). Cyclization with sodium ethoxide or sodium hydride in ether gave the desired 2-phenyl-cyclohexane-1,3-dione (V), m.p. 160—161°. (*Anal.* Calcd. for $C_{12}H_{12}O_2$: C, 76.6; H, 6.4. Found: C, 76.8; H, 6.4). 2-Phenyl-3-methoxy-cyclohex-2-enone (VIa) resulted from the reaction of (V) with diazomethane, m.p. 96—98°; λ_{max} 270 mμ; $E = 14,500$. (*Anal.* Calcd. for $C_{13}H_{14}O_2$: C, 77.2; H, 6.9; Found: C, 76.9; H, 6.9). The enol ethyl ether (VIb), m.p. 102°. (*Anal.* Calcd. for $C_{14}H_{16}O_2$: C, 77.8; H, 7.4; Found: C, 77.6; H, 7.5) was obtained by treatment with ethyl orthoformate in ethanol.

Lithium aluminum hydride converted VIa into 2-phenyl-cyclohex-2-enone (VII), m.p. 95—96°, which was identical with an authentic sample[7].

Employing benzyl cyanide and ethyl α,α-dimethyl-glutarate, ethyl 2,2-dimethyl-5-keto-6-phenylcaproate (IVb) was prepared, b.p. 145° at 1 mm. (*Anal.* Calcd. for $C_{16}H_{22}O_3$: C, 73.3; H, 8.4. Found: C, 73.2; H, 8.5). Cyclization with sodium hydride in toluene gave 2-phenyl-6,6-dimethyl-cyclohexane-1,3-dione (Vb), m.p. 210°. (*Anal.* Calcd. for $C_{14}H_{16}O_2$: C, 77.8; H, 7.4. Found: C, 78.1; H, 7.7). (Vb) reacted with diazomethane to give a mixture of 2-phenyl-3-methoxy-6,6-dimethyl-cyclohex-2-enone (VIII), m.p. 134—135°; λ_{max} 268 mμ; $E = 13,700$ (*Anal.* Calcd. for $C_{15}H_{18}O_2$: C, 78.3; H, 7.8. Found: C, 78.3; H, 8.0) and 2-phenyl-3-methoxy-4,4-dimethyl-cyclohex-2-enone (IX), m.p. 37°; λ_{max} 263 mμ; $E = 12,000$. (*Anal.* Calcd. for $C_{15}H_{18}O_2$: C, 78.3; H, 7.8. Found: C, 78.4; H, 8.0).

(IX) is hydrolyzed more slowly than (VIII) by boiling 5% aqueous sodium hydroxide[8] and correspondingly shows a hypsochromic shift in the ultraviolet spectrum.

Reduction of (VIII) gave 2-phenyl-4,4-dimethyl-cyclohex-2-enone (X) as an oil which was characterized as the dinitrophenylhydrazone (red), m.p. 172—174°. (*Anal.* Calcd. for $C_{20}H_{20}O_4N_4$: C, 63.2; H, 5.3. Found: C, 63.1; H, 5.3. λ_{max} 380 mμ; $E = 63,000$), and reduction of (IX)-2-

I

II

$$\underset{III}{C_6H_5.\overset{\overset{\displaystyle CN}{|}}{CH}-CO.(CH_2)_3.COOC_2H_5} \xrightarrow{2\,steps} \underset{IV}{C_6H_5.CH_2.CO.(CH_2)_2.\overset{\overset{\displaystyle R}{|}}{\underset{\underset{\displaystyle R}{|}}{C}}.COOEt}$$

IV

a : R=H
b : R=CH₃

V

a : R=H
b : R=CH₃

VI

a : R₁=OCH₃
b : R₁=OC₂H₅

VII

VIII

X

IX

XI

phenyl-6,6-dimethyl-cyclohex-2-enone (XI) as an oil, dinitrophenyl-hydrazone (orange), m.p. 161—162⁰. (*Anal.* Calcd. for $C_{20}H_{20}O_4N_4$: C, 63.2; H, 5.3. Found: C, 63.3; H, 5.2) λ_{max} 375 mμ; $E = 67,000$. As expected (XI) shows a hypsochromic shift (cf. the spectrum of the dinitrophenylhydrazone of 2-phenyl-cyclohex-2-enone[7] for which λ_{max} 380 mμ; $E = 25,800$ has been reported).

M. BORNSTEIN
R. PAPPO
*Weizmann Institute of Science,
Rehovot*

J. SZMUSZKOVICZ
Israeli Ministry of Defence

REFERENCES

1. Frank and Hall Jr., 1950, *J. Amer. Chem. Soc.*, 72, 1645.
2. Seifert and Schinz, 1951, *Helv. Chim. Acta*, 34, 728.
3. Blanchard and Goering, 1951, *J. Amer. Chem. Soc.*, 73, 5863.
4. Nystrom and Brown, 1947, *ibid.*, 69, 1198.
5. J. Szmuszkovicz, unpublished results.
6. Butz, Davis and Gaddis, 1947, *J. Org. Chem.*, 12, 122.
7. Ginsburg and Pappo, 1951, *J. Chem. Soc.*, 516.
8. Von E. Doering and Knox, 1951, *J. Amer. Chem. Soc.*, 73, 828.

Estrone Inactivation in vitro.
Estrinase Distribution in Rat Liver Cell Fractions

The inactivation of estrogens by liver *in vivo* and *in vitro* in poikilo- and homio-thermal animals as well as in plants has been studied in this laboratory for many years. It was concluded that an enzymatic process (probably of specific character, named estrinase) is responsible for the inactivation mechanism[1-5].

The object of the present experiments was to establish by differential centrigufation which fractions of the cell-free rat liver extracts contained the inactivating enzyme system.

The homogenization and the centrifugation were performed in a cold room (0⁰—4⁰C). Homogenization was carried out in a test tube in 0.25 M sucrose + 4% nicotinamide[6] using a motor driven plastic homogenizer. The liver cell fractions (nuclei mitochondria and supernatant) were isolated in a Sorvall angle-centrifuge (Type SS IA) following the procedure of Schneider and Hogeboom[7].

Fractions from 10% homogenate, equal to 1 g weight of tissue, were incubated in 1/15 M phosphate buffer pH 7.5 with 100 γ estrone. Aliquots were placed into flasks, containing 50 ml 96% ethanol, immediately (T_0) and after 4 hrs shaking (T_4) in an incubator at 37⁰C. From the difference of the two readings obtained after extraction and purification of estrogen or metabolites, the percentage inactivation was calculated[8].

The determination of estrogens in rat liver homogenates by fluorometry —details of which will be given elsewhere—has been found to be a convenient and sensitive method[9]. The disappearance of fluorescence has been found to parallel biological inactivation after four hour incubation periods at 37⁰C as proved with control experiments with Allen-Doisy bioassay (Table I).

TABLE I

Exp. No.	Estrone added to homogenate γ	Estrone found at Time T_0 γ		Estrone found at Time T_4 γ		Inactivation % $\dfrac{T_0\text{-}T_4 \times 100}{T_0}$	
		Fluorometry	Bioassay*	Fluorometry	Bioassay*	Fluorometry	Bioassay*
1	0.0	0.0	0.2	0.0	0.0	0	0
2	0.0	0.0	0.0	0.0	0.0	0	0
3	10.0	7.5	8.0	1.5	1.0	80	88
4	10.0	8.8	8.0	2.0	2.0	77	75
5	10.0	7.5	8.0	1.5	2.0	80	75
6	10.0	9.5	8.0	2.5	1.0	74	88

*Allen-Doisy. 10 mμ = 1 γ estrone.

The results (Table II) of the centrifugation experiments show that the inactivating enzyme system was in the supernatant fraction, and that the nuclei and mitochondria do not participate to any considerable extent in the inactivation of estrone. The presence of the inactivating system in one cell fraction will help in the elucidation of the mechanism of inactivation.

TABLE II
Estrone Inactivation by homogenate and fractions
(10 γ estrone added in every experiment)

Exp. No.		Estrone found at time T_0	Estrone found at time T_4	Inactivation % $\dfrac{T_0\text{-}T_4 \times 100}{T_0}$
1	a) Total homogenate	10.5	2.5	76
	b) Nuclei	8.5	8.0	6
	c) Mitochondria	10.0	8.5	15
	d) Supernatant	9.0	2.5	72
2	a) Total homogenate	7.5	1.0	86
	b) Nuclei + Mitochondria	8.5	7.5	12
	c) Supernatant	7.5	1.5	80
3	a) Total homogenate	7.5	2.5	66
	b) Supernatant	9.0	2.5	72
	c) Nuclei + Mitochondria	7.0	6.5	7
	d) Nuclei + Mitochondria + Supernatant	7.5	3.0	60

Total homogenate and fraction in each case is equivalent to 100 mg tissue.

GIDEON RUMMELSBURG
*Hormone Research Laboratory,
Hebrew University-Hadassah Medical School,
Jerusalem.*

REFERENCES

1. Zondek, B., 1934, *Skandinav. Arch. F. Physiol.*, 70, 133 and *Lancet*, 227, 356.
2. Zondek, B. and v. Euler, H., 1934, *Biochem. Z.*, 271, 64
3. Zondek, B. and Sklow, J., 1942, *Proc. Soc. Exp. Biol. & Med.*, 49, 629.

4. ZONDEK, B., SULMAN, F. G., SKLOW, J., 1943, *Endocrinology*, 33, 333.
5. ZONDEK, B. and FINKELSTEIN, M., 1945, *Endocrinology*, 36, 291.
6. DE MEIO, R. H., RAKOFF, A. E., CANTAROW, A. and PASCHKIS, E. K., 1948, *Endocrinology*, 43, 97.
7. SCHNEIDER, W. C. and HOGEBOOM, G. H., 1950, *J. biol. Chem.*, 183, 123.
8. GOLDENBERG, CHANA, 1950, M.Sc. Thesis, The Hebrew University, Jerusalem.
9. FINKELSTEIN, M. and GOLDENBERG, CH., 1951, *Bull. Res. Counc. of Israel*, 1, No. 1-2, 154.

Received December 29, 1952.

Appendix to "On the Solubility of Sodium Chloride in Dead Sea Brines"[1]

It has been brought to our attention by Mr. H. Z. Littman that the solubility of sodium chloride in Dead Sea brines has been previously determined by him in connection with an investigation carried out by Dr. R. Bloch and the late Prof. L. Farkas.

In an unplublished report to the Palestine Potash Company Ltd., the solubility of salt in surface brine was given as follows:

Solubility of sodium chloride in natural Dead Sea brine

Temperature (°C)	g Na Cl dissolved in 1 litre brine $d^{25}_4 = 1.191$
10	58
25	62
40	66

F. YARON
I. SCHNERB
Research Council of Israel

REFERENCE

1. SCHNERB, I. and YARON, F., 1952, *Bull. Res. Counc. of Israel*, 2, 197.

Papers presented in the Section of Organic Chemistry

Observations on Polar C = C Double Bonds

The application of the method of molecular or bitals to substances of fulvene type

has given results which are generally in accord with the previous experience of organic chemistry, and has shown that this experience is due to the polar character of the semicyclic double bond. Amongst the results, there is, however, one which is somewhat beyond the expectations of the organic chemist, viz. the electron distribution in vinyl-dibenzofulvene[1]:
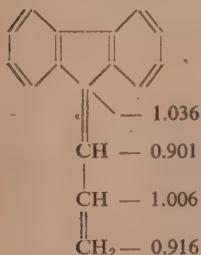

(the figures are fractions of a total negative charge).

In this compound, the polarity remains concentrated mainly in the semicyclic double bond of dibenzofulvene and is only slightly affected by the conjugation with the vinyl group. Indeed, subsequent experiments with cinnamylidene-fluorene (I) and lithium aluminium hydride, which is considered a specific reagent for the reduction of *polar* double bonds[2,3], have shown that only the semicyclic double bond is attacked and a lithium derivative of 9-cinnamyl-fluorene (II) is formed[4]. It appeared interesting to study the response of (I) to another reagent for polar double bonds, lithium phenyl, which has already been applied to fulvene systems[5,6].

(I) gives with lithium phenyl a deeply coloured lithium derivative, indicating that the lithium atom has indeed attacked the most negative atom of the system, the C^9 - atom of the fluorene nucleus. Hydrolysis gave a hydrocarbon $C_{28}H_{22}$ of m.p. 173° (from *iso* propanol) (*Anal.* Calcd. for $C_{28}H_{22}$: C, 93.8; H, 6.2; mol. wt., 358. Found: C, 93.6; H, 6.2; mol. wt., 374 (in camphor)), for

which two formulae are possible (III, IV), in which the asterisk marks the hydrogen atom derived from the lithium of the metal-organic compound.
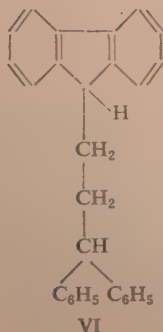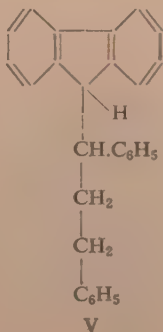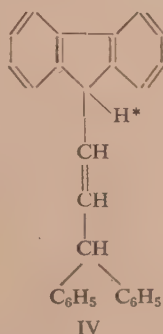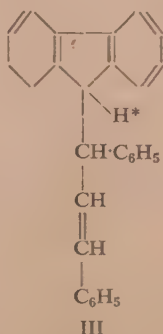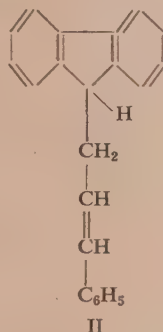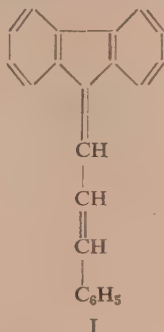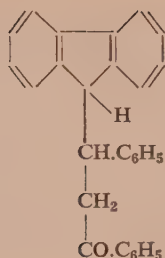
VII
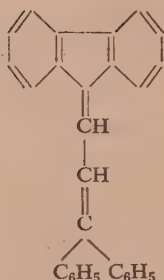
VIII
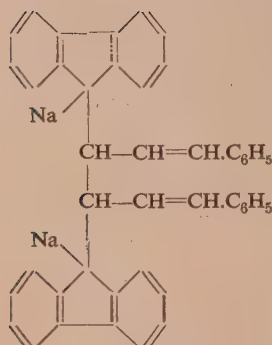
IX
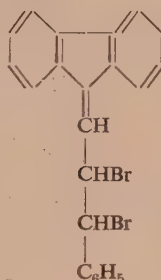
X
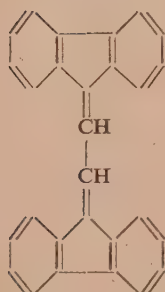
XI
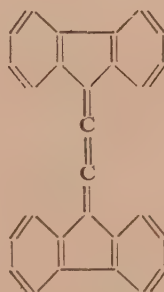
XII

A decision was made by the synthesis of the dihydro-derivatives (V, VI) of (III) and (IV) and their comparison with the hydrocarbon $C_{28}H_{24}$, which was obtained by catalytic hydrogenation of $C_{28}H_{22}$ (m.p. 102°, from glacial acetic acid. (*Anal.* Calcd. for $C_{28}H_{24}$: C, 93.3; H, 6.7. Found: C, 93.1; H, 6.7). The compound was identical with (VI); $C_{28}H_{22}$ therefore, had structure (IV).

For the synthesis of 1,3-diphenyl-1-(9'-fluorenyl)-propane (V) the corresponding ketone

(VII)[7,8] was reduced by the method of Huang-Minlon[9]). M. p. 113°, from cyclohexane. (*Anal.* Calcd. for $C_{28}H_{24}$: C, 93.3; H, 6.7. Found: C, 93.3; H, 6.7).

3,3-Diphenyl-1-(9'-fluorenyl)-propane (VI) was obtained by catalytic hydrogenation of 1-biphenylene-4,4-diphenyl-butadiene (VIII)[10]). M.p. 102°, from *iso*propanol. (*Anal.* Calcd. for $C_{28}H_{24}$: C, 93.3; H, 6.7. Found: C, 93.2: H, 6.9).

In the reaction with lithium phenyl, therefore — in contradistinction with that with lithium aluminium hydride — the polarity induced in the vinyl group is the determining factor of the reaction.

In this connection, it is recalled that sodium metal attacks (I) to give (IX), i.e. a derivative of 2,3-distyryl-1, 4-dibiphenylene-butane[11]). On the other hand, bromination of (I) is restricted to the vinyl group, (X) being formed[12]). It has been known for some time — although no explanation has yet been offered for this fact — that the semicyclic double bond in fulvenes is surprisingly resistant to halogen molecules in non-polar solvents[13]).

It is also worthy of note that in dibiphenylene-butadiene (XI) and dibiphenylene-butatriene (XII) the semicyclic double bonds react with lithium aluminium hydride independently of each other[14].

ERNST D. BERGMANN
*Scientific Department,
Ministry of Defence*

REFERENCES

1. PULLMANN, B. and PULLMANN, A., 1952, *Les Theories Electroniques de la Chimie Organique*, Mason et Cie, Paris p. 403.
2. NYSTROM, R. F. and BROWN, W. G., 1947, *J. Amer. Chem. Soc.*, **69**, 1197, 2548; 1948, 70, 3738.
3. BROWN, W. G., *Organic Reactions*, 1951, Wiley, New York, **6**, 469.
4. LAVIE, D. and BERGMANN, E. D., 1951, *Bull. Soc. Chim. France*, **18**, 250.
5. ZIEGLER, K. and SCHAEFER, W., 1934, *Ann.*, **511**, 101.
6. FUSON, R. C., DE WALD, H. A. and GAERTNER R., 1951, *J. Org. Chem.*, **16**, 26.
7. PINCK, L. A. and HILBERT, G. E., 1946, *J. Amer. Chem. Soc.*, **68**, 2014.
8. TUCKER, S. H. and WHALLEY, M., 1949, *J. Chem. Soc.*, **50**.
9. HUANG-MINLON, 1949, *J. Amer. Chem. Soc.*, **71**, 3301.
10. BERGMANN, E. D. and FISCHER, E., 1950, *Bull. Soc. Chim. France*, **17**, 1084.
11. SCHLENK, W. and BERGMANN, E., 1928, *Ann.*, **463**, 65.
12. THIELE, J. and HENLE, F., 1906, *Ann*, **347**, 304.
13. BERGMANN, E. and V. CHRISTIANI, A., 1930, *Ber. Dtsch. Chem. Ges.*, **63**, 2559.
14. LAVIE, D. and BERGMANN, E. D., 1953, *J. Org. Chem.*, **18**, (in press).

Synthesis of 2,9-Diketo-1, 2, 3, 4, 4a, 9, 10, 10a-octahydrophenanthrene

In order to widen the scope of the phenanthrene synthesis based on the Michael condensation of suitably substituted 2-phenyl-cyclohexenones[1], a practical way for the preparation of 3-phenyl-cyclohexen-6-one (I) was sought, an analogue of the known 2-phenyl-cyclohexen-3-[1] and -6-ones (II, III).[2] This note reports a method which is based
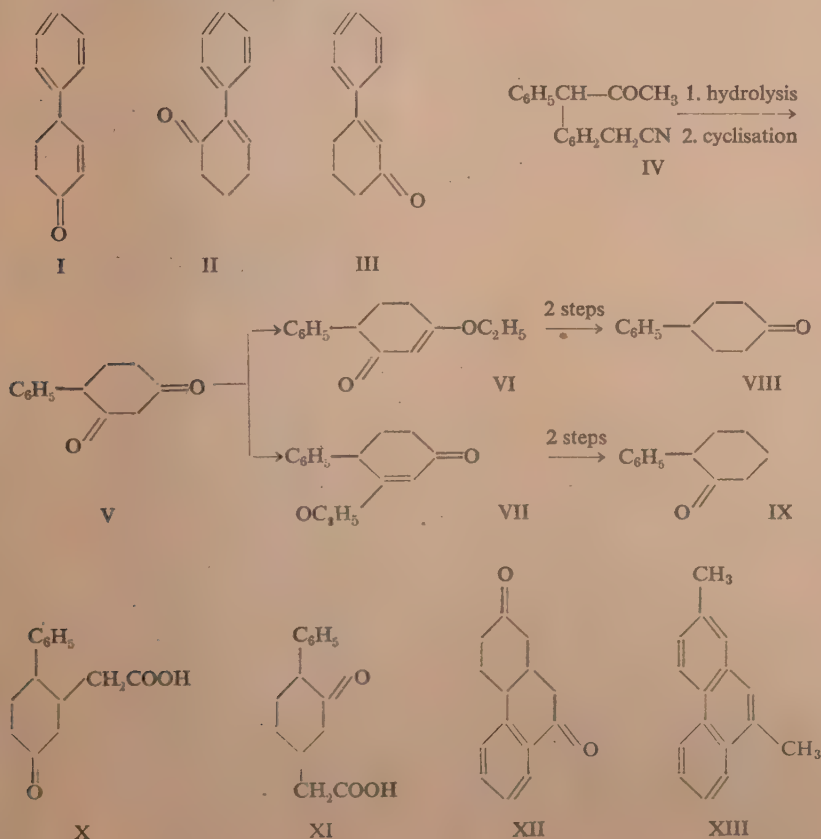
on the easily available starting materials acrylo-nitrile and phenylacetone. The two compounds reacted with each other under specified conditions[3] to give γ-phenyl-γ-aceto-butyronitrile (IV), b.p. 124—126° at 0.1 mm, $n_D^{25}=1.4250$. (Anal. Calcd. for $C_{12}H_{13}NO$: N, 7.5. Found: N, 7.2). Semicarbazone, m.p. 163.5—165.5°.

The methyl ester of the corresponding acid was cyclised by means of sodium alkoxide to 4-phenyl-cyclohexane-1,3-dione (V), m.p. 113°. (Anal. Calcd. for $C_{12}H_{12}O_2$: C, 76.6; H, 6.4. Found: C, 76.6; H, 6.7). The structure is supported by the violet colour reaction with aqueous ferric chloride solution.

The diketone (V) was converted to a mixture of enol ethers (VI, VII) by azeotropic distillation with ethanol and benzene in presence of p-toluene-sulphonic acid (b.p. 139—141° at 0.5 mm; $n_D^{25}=$ 1.5650; λ_{max} 250 mμ; log ε 4.53) and the mixture reduced by means of lithium aluminium hydride[4]. Thus a mixture of phenyl-cyclohexenones was

obtained; b.p. 122—123° at 1 mm; $n_D^{25}=$ 1.550. (Anal. Calcd. for $C_{12}H_{12}O$: C, 83.7; H, 7.0. Found: C, 83.6; H, 7.4), which, upon hydrogenation, afforded 4- and 2-phenylcyclohexanone (VIII, IX)[5,6] in a ratio of about 1:1. The two known ketones were separated by fractional crystallisation of their semicarbazones. The lithium aluminium hydride product contained thus undoubtedly the desired ketone (I).

The mixture of phenylcyclohexenones reacted smoothly with dibenzyl malonate in presence of potassium tert. butoxide. Hydrolysis and partial decarboxylation gave two acids, which have been identified as 4-phenyl-cyclohexanone-3-acetic acid (X), m.p. 166.5—168° (Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72.4; H, 6.9. Found: C, 72.5; H, 7.0) and 2-phenyl-cyclohexanone-5-acetic acid (XI), m.p. 134.5—135.5° (Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72.4; H, 6.9. Found: C, 72.3; H, 6.8), respectively, by reduction (Huang-Minlon's method) to trans-2-phenyl-cyclohexylacetic acid[7] and trans-4-phenyl-cyclohexylacetic acid[5]. It is worthy of

$C_6H_5CH\!-\!COCH_3$
$\underset{C_6H_5CH_2CN}{|}$ $\xrightarrow[\text{2. cyclisation}]{\text{1. hydrolysis}}$

IV

I   II   III

$C_6H_5\!-\!\overset{\diagup}{\underset{O}{\diagdown}}\!=\!O$ —

$\rightarrow C_6H_5\!-\!\text{—}\!-OC_2H_5$ $\xrightarrow{\text{2 steps}}$ $C_6H_5\!-\!\text{—}\!=\!O$

VI   VIII

$\rightarrow C_6H_5\!-\!\text{—}\!=\!O$ $\xrightarrow{\text{2 steps}}$ $C_6H_5\!-\!\text{—}$

OC_3H_5 VII   IX

V

$\underset{C_6H_5}{\overset{C_6H_5}{|}}\!\diagdown\!CH_2COOH$ $\underset{CH_2COOH}{\overset{C_6H_5}{|}}\!\diagup\!O$

X   XI   XII   XIII

note that the Michael condensation with dibenzyl malonate affords *trans*-structures.

(X) was cyclised with sulphuric acid to 2,9-di-keto-1, 2, 3, 4, 4a, 9, 10, 10a-octahydrophenan-threne (XII), m.p. 166—168°; λ_{max} 249, 289 m μ; log ε 4.13; 3.12. (*Anal.* Calcd. for $C_{14}N_{14}O_2$; C, 78.5; H, 6.5. Found: C, 78.4; H, 6.7); dioxime, m.p. 210—212°; diketal with ethylene-glycol, m.p. 156.5—158°.

For further proof, (XII) was converted by successive reaction with methylmagnesium iodide, dehydration and dehydrogenation, into the known 2,9-dimethyl-phenanthrene (XIII)[8].

The ways in which the method described here can be applied and extended are now being studied.

J. SZMUSZKOVICZ
E. D. BERGMANN
Scientific Department
Ministry of Defence

REFERENCES

1. PAPPO, R. and GINSBURG, D., 1951, *Bull. Res. Counc. of Israel*, 1, No. 3, 121; 1951, *J. Chem. Soc.*, 516, 938.
2. ABDULLAH, 1935, *Chem. Zentr.*, 106, I, 3926.
3. MIKESKA, L. A., U. S. Patent, 2,461, 366 (*Chem. Abstr.*, 43, 4689 (1949)).
4. FRANK, R. L. and HALL, H. K. Jr., 1950, *J. Amer. Chem. Soc.*, 72, 1645; BLANCHARD, J. P. and GOFRING, H. L., 1951, *ibid.*, 73, 5863; BORNSTEIN, M., PAPPO, R. and SZMUSZKOVICZ, J., (unpublished results).
5. FIESER, L. F., LEFFLER M. T. and co-worker, 1948, *J. Amer. Chem. Soc.*, 70, 3186.
6. NEWMAN, M. S. and FARBMAN, M. D., 1944, *ibid.*, 66, 1550.
7. BACHMANN, W. E. and FORNEFELD, E. J., 1950, *ibid.*, 72, 5529.
8. HAWORTH, R. D., MARTIN, C. R. and SCHELDRICK, G., 1934, *J. Chem. Soc.*, 454.

5-Fluoro-2,4-dinitro-aniline and its Derivatives, New Reagents for Amino-acids and Peptides

The easy replaceability of the fluorine atom in 2,4-dinitro-fluorobenzene has made it a valuable reagent for amino-acids and peptides[1], especially if the replacement reaction is followed by chromatography[2]. The success of this method appeared to warrant an extension into several directions: introduction of polar groups into the aromatic nucleus is likely to lead to better-crystallised and more easily identified derivatives; if the polar group is an amino group, the derivatives obtained might be transformed into azo-dyes, easily accessible to quantitative spectrophotometric determination, etc.

5-Fluoro-2,4-dinitro-acetanilide and 5-fluoro-2,4-dinitro-aniline have been prepared for these purposes. 3-Fluoronitrobenzene, obtained by Schiemann's reaction from 3-nitroaniline[3], was catalytically hydrogenated and 3-fluoro-aniline[4], so obtained, acetylated and nitrated at a temperature not exceeding 0°. 5-Fluoro-2,4-dinitro-acetanilide was thus formed in 72% yield; m.p. 119° (from alcohol) (*Anal.* Calcd. for $C_8H_6O_4N_3F$: N, 17.3. Found: N, 17.4; 17.6). Hydrolysis

with 20% hydrochloric acid gave 5-fluoro-2,4-dinitro-aniline, m.p. 186° (from alcohol), which had been obtained before by a different method[5].

The following derivatives of amino-acids have been obtained in the usual manner[1] in good yield and have proven to be well-crystallized compounds which can be used for the identification of the amino-acids:

$N-$(2,4-Dinitro-5-acetamido-phenyl)-glycine, m. p. 243° (from alcohol).

$N-$(2,4-Dinitro-5-acetamido-phenyl)-L-phenyla-lanine, m.p. 208° (from aqueous alcohol).

$N-$(2,4-Dinitro-5-acetamido-phenyl)-DL-methio-nine, m.p. 252° (dec.) (from glacial acetic acid) (*Anal.* Calcd. for $C_{13}H_{16}O_6N_4S+H_2O$: C, 40.0; H, 4.6; N, 14.3. Found: C, 40.8; H, 3.9; N, 13.5).

$N-$(2,4-Dinitro-5-acetamido-phenyl)-DL-serine, m.p. 204° (from alcohol).

O, N-(2,4-Dinitro-5-acetamido-phenyl)-(2,4-dini-ro-5-amino-phenyl)-L-tyrosine, m.p. 254° (dec.) (from acetone)). (*Anal.* Calcd. for $C_{23}H_{19}O_{12}N_7$: C, 47.2; H, 3.2; N, 16.8. Found: C, 46.2; H, 3.1; N, 16.9). In this reaction, one of the acetyl groups is split off which seems to happen with surprising ease also with 2,4-dinitro-5-fluoroace-tanilide itself.

ERNST D. BERGMANN
MICHAEL BENTOV
Scientific Department
Ministry of Defence

REFERENCES

1. SANGER, 1945, *Biochem. J.*, 39, 507; PORTER and SANGER, 1948; *Biochem. J.*, 42, 287.
2. *Cf.* JUTISZ M., 1952, *Bull. Soc. Chim. France*, 19, 821.
3. SCHIEMANN and PILLARSKY, 1929, *Ber.*, 62, 3035.
4. SWARTS 1913, *Rec. Trav. Chim. Pays-Bas*, 32, 65.
5. SWARTS, 1916, *Rec. Trav. Chim. Pays-Bas*, 35, 161.

The Synthesis of New Macrocyclic Systems

2,2'-Bis-(bromomethyl)-biphenyl(I) reacts with lithium phenyl to give 9,10-dihydrophenan-threne (III),[1,2,3,4] according to Wittig and Witt[5] via the intermediate (II). It seemed interesting to study the behaviour of 2,2'-bis-(bromomethyl)-diphenylmethane and- dibenzyl (IVb, Vb): if the greater mobility of these molecules will not prevent cyclisation reactions, seven- and eight-membered ring systems may thus become available.

Reduction of the esters of diphenylmethane-and dibenzyl-2,2'-dicarboxylic acid (VI, VII) with lithium aluminum hydride, led to 2,2'-bis-(hydroxymethyl)-diphenylmethane (IVa) (m.p. 158°, from alcohol; (*Anal.* Calcd. for $C_{15}H_{16}O_2$: C, 79.0; H, 7.0. Found: C, 78.8; H, 7.0) and 2,2'-bis-(hydroxymethyl)-dibenzyl (Va) (m.p. 151°, from alcohol); (*Anal.* Calcd. for $C_{16}H_{18}O_2$: C, 79.3; H, 7.4. Found: C, 80.5; H, 7.6).

The corresponding bromides were prepared by means of phosphorus tribromide. (IVb) had

CH₂Br
CH₂Br

I

CH₂Br
CH₂Li

II

III

CH₂X
CH₂
CH₂X

IV, a: X = OH
b: X = Br

CH₂X
CH₂
CH₂
CH₂X

V, a: X = OH
b: X = Br

COOH
CH₂
COOH

VI

COOH
CH₂
CH₂
COOH

VII

CH₂—CH₂
CH₂   CH₂
CH₂—CH₂

VIII

CH₂—CH₂
CH₂   CH₂
CH₂   CH₂
CH₂—CH₂

IX

CH = CH
CH   CH
‖   ‖
CH   CH
CH = CH

X
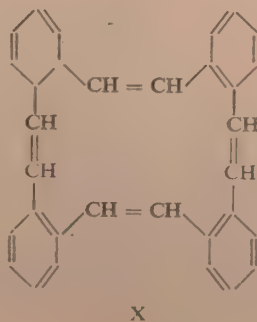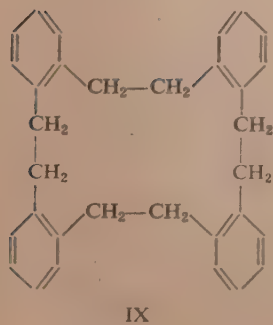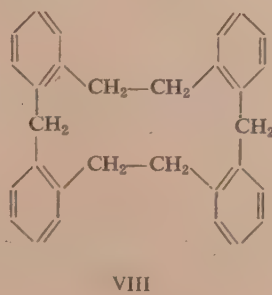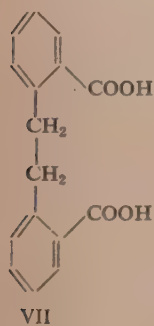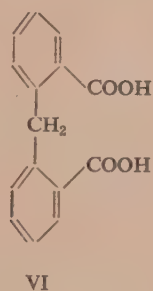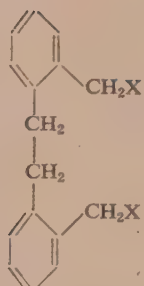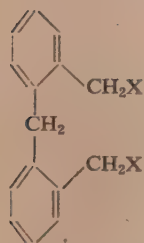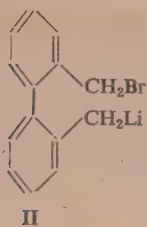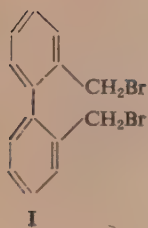
m.p. 93—94° (from benzene) (*Anal.* Calcd. for $C_{15}H_{14}Br_2$: Br, 45.1. Found: Br, 45.1). (Vb) had m.p. 137 — 138° (from benzene) (*Anal.* Calcd. for $C_{16}H_{16}Br_2$: Br, 43.4. Found: Br, 43.1). Their reactions with lithium phenyl gave partly amorphous, polymeric material — which is not unexpected in such a reaction of bifunctional molecules — and to about 50% of the theory crystalline hydrocarbons $C_{30}H_{28}$ and $C_{32}H_{32}$ respectively, i.e. *bimolecular* condensation products of the starting materials.

The hydrocarbon $C_{30}H_{28}$ (m.p. 195°, from benzene; *Anal.* Calcd. for $C_{30}H_{28}$: C, 92.7; H, 7.3; mol. wt., 388. Found: C, 92,5; H, 7.4; mol. wt., 397) can only have the formula (VIII) of an 1, 2, 4, 5, 8, 9, 11, 12-tetrabenzo-cyclotetradeca-1, 4, 8, 11-tetraene. For the hydrocarbon $C_{32}H_{32}$ (m.p. 205°, from benzene; *Anal.* Calcd. for $C_{32}H_{32}$: C, 92.3; H, 7.7; mol. wt., 416. Found: C, 92.3; H, 7.8; mol. wt., 430) the structure of an 1, 2, 5, 6, 9, 10, 13, 14-tetrabenzo-cyclohexadeca-1, 5, 9, 13-tetraene has been proven by treatment with N-bromosuccinimide, which gives a tetrabromo-derivative of m.p, 235° (dec.) (from isopropyl alcohol) (*Anal.* Calcd. for $C_{32}H_{28}Br_4$: Br, 43.4. Found: Br, 43.0). Dehydrohalogenation of the latter leads to a hydrocarbon $C_{32}H_{28}$, which consequently is to be formulated as 1, 2, 5, 6, 9, 10, 13, 14-tetrabenzo-cyclohexadeca-1, 3, 5, 7, 9, 11, 13, 15-octaene (X), m.p. 267—268° (from petroleum ether) (*Anal.* Calcd. for $C_{32}H_{28}$: C, 94.1; H, 5.9. Found: C, 94.1; H, 6.0). It is interesting that this substance is not coloured in spite of the accumulation of conjugated double bonds.

The successive reaction with N-bromosuccinimide and dehydrohalogenation can be considered as a general method for the identification of a dibenzyl system.[6,7,8,9]

Molecular models show that the three newly prepared macrocyclic hydrocarbons are completely strainless. The principles of their synthesis may shed some light on the pathway on which the living cell arrives at such macrocyclic systems as muscone, civetone or dextro-tubocurarine chloride.

ERNST D. BERGMANN
ZVI PELCHOWICZ
Scientific Department,
Ministry of Defence

REFERENCES

1. HALL and TURNER, 1949, *Nature*, 163, 537.
2. HALL, LESSLIE and TURNER, 1952, *J. Chem. Soc.*, 711.
3. HALL and TURNER, 1951, *J. Chem. Soc.*, 3072.
4. BERGMANN, E. and SZMUSZKOVICZ, 1951, *J. Amer. Chem. Soc.*, 73, 5153.
5. WITTIG and WITT, 1941, *Ber.*, 74, 1474.
6. BARNES, 1948, *J. Amer. Chem. Soc.*, 70, 146.
7. BERGMANN, E.D., *et al.*, 1951, *Bull. Soc. Chim. France*, 18, 684.
8. BERGMANN, E. D., 1952, *J. Amer. Chem. Soc.*, 74, 1075.
9. BERGMANN, E. D., CRANE and FUOSS, 1952, *J. Amer. Chem. Soc.*, 74, (in press).

Stereochemical Relationships in the Chloromycetin Series

When ethyl *erythro-N-(p-nitrobenzylidene)-p-ni-trophenylserinate* (V) is treated with an excess of ethyl glycinate, a curious configurational change takes place, leading to ethyl *threo-p-nitrophenyl-serinate* (I)[1,2], i.e. to a substance which has the configuration of chloromycetin. One might ascribe this inversion to the existence of a tautomeric equilibrium between (V) and the isomeric oxazolidine (VI, R=H)[3]; it would then be analogous to the acyl migration in monoacylated β-hydroxyamines for which also a common intermediate of oxazolidine structure has been assumed[4,5].

It seemed of interest to study the question whether oxazolidines derived from such diastereomeric β-hydroxyamines, as, e.g., the diastereomeric *p*-nitrophenylserines, are structurally and configurationally stable. This has been made possible by the following observation: the condensation of the ethyl *p*-nitrophenylserinates (I, IV) with *p*-nitrobenzaldehyde leads not to oxazolidines, but to Schiff bases (II, V); but the acetylation of the latter with boiling acetic anhydride is accompanied by tautomerisation and gives the diastereomeric ethyl 2,5-di-(*p*-nitrophenyl)-3 - acetyl - oxazolidine - 4 - carboxylates (III, VI), *which are stable compounds*. In this type of oxazolidines, therefore, no configurational change takes place under the experimental conditions employed.

The structure of the four substances (II, III, V, VI) has been established on the basis of their

I. $NO_2.C_6H_4$——C————C——$COOC_2H_5$
with OH and H on the first C, H and NH_2 on the second C.

II. $NO_2.C_6H_4$——C————C——$COOC_2H_5$
with OH and H on the first C, H and N on the second C; N double bonded to CH, CH bonded to $C_6H_4.NO_2$.

III. $NO_2.C_6H_4$——C————C——$COOC_2H_5$
with O and H on the first C, H and N. R on the second C; forming ring through CH, C_6H_4, NO_2.

IV. NO$_2$.C$_6$H$_4$——C————C——COOC$_2$H$_5$
with H above each C, OH and NH$_2$ below the carbons

V. NO$_2$.C$_6$H$_4$——C————C——COOC$_2$H$_5$
with H above each C, OH below first C, N below second C, N=CH, C$_6$H$_4$.NO$_2$

VI. NO$_2$.C$_6$H$_4$——C————C——COOC$_2$H$_5$
with H above each C, O below first C, N.R below second C, CH bridging, C$_6$H$_4$.NO$_2$

ultraviolet and infrared spectra. The Schiff bases (II) and (V) absorb at 2700 and 2710 A, respectively, whilst the maximum of the acetyl derivatives (III, VI) lies at 2655 A, due to the shorter conjugated system. These results are in accord with the spectra of N-(p-nitrobenzylidene)-ethanolamine (2750 A) and 2-(m-nitrophenyl)-2, 4, 5, 5-tetramethyloxazolidine (2650 A)[6].

In the infrared spectrum of (II) and (V)[7,8,9], the C=N absorption and that of the hydroxyl group are the characteristic features, whilst the spectra of (III) and (VI) show no sign of the C=N absorption, but instead the characteristic band group of the oxazolidine system and (at 1660 cm^{-1}) the absorption of a disubstituted amide[7,10].

The *erythro*-Schiff base (V) has been described before [8,9], the *threo*-isomer (II) is formed when ethyl *threo-p*-nitrophenylserinate (I) is condensed azeotropically with *p*-nitrobenzaldehyde[11]; m.p. 120° (from *iso*propyl alcohol). Acetylation of the erythro-form with acetic anhydride gave (VI), m.p. 172° (*Anal.* Calcd. for C$_{20}$H$_{19}$O$_8$N$_3$: C, 56.0; H, 4.4; N, 9.8; OC$_2$H$_5$, 10.5. Found: C, 56.0; H, 4.7; N, 9.9; OC$_2$H$_5$, 10.0%). Also the acetyl derivative (III) of the *threo*-form melted at 172° (from *iso*propyl alcohol), but depressed considerably the melting point of the isomer. (*Anal.* Found: C, 55.9; H, 4.7; N, 10.0; OC$_2$H$_5$, 10.2%).

There are some cases in which Schiff bases of β-hydroxyamines have been known to give 3-acyl-oxazolidines on acylation, but this is one of the few cases in which diastereometric oxazolidines have been derived from open-chain aminoalcohols. McCasland and Horswill[12] have described the analogous pair derived from the cis-trans-isomeric 2-aminocyclohexanols, and in the thiazolidine series, one analogous case is known in the condensation of the two thiothreonines with ethyl benzylpenaldate[13].

ERNST D. BERGMANN
HILLEL BENDAS
Scientific Department
Ministry of Defence

REFERENCES

1. ELPHIMOFF-FELKIN, FELKIN and WELVART, 1952, *Compt. Rend.*, 234, 1564.
2. BERGMANN E., BENDAS and RESNICK, 1952, *J. Chem. Soc.*, (in press).
3. BERGMANN E., GIL-AV and PINCHAS, 1952, *J. Amer. Soc.*, (in press).
4. MCCASLAND, CLARK and CARTER, 1949, *J. Amer. Chem. Soc.*, 71, 637.
5. WILEY and BENNETT, 1949, *Chem. Rev.*, 44, 447.
6. BERGMANN E., HIRSCHBERG, PINCHAS and ZIMKIN, 1952, *Rec. Trav. Chim. Pays-Bas*, 71, 192.
7. BERGMANN E., ZIMKIN and PINCHAS, 1952, *Rec. Trav. Chim. Pays-Bas*, 71, 188; 231, 361.
8. BERGMANN. E., BENDAS and GENAS, 1950, *Compt. Rend.*, 231, 361.
9. BERGMANN E., BENDAS and TAUB, 1951, *J. Chem. Soc.*, 2673.
10. RICHARDS and THOMPSON, 1947, *J. Chem. Soc.*, 1248.
11. *Cf.* HOLLAND and NAYLER, 1952, *Chem. and Ind.*, 518.
12. MCCASLAND and HORSWILL, 1951, *J. Amer. Chem. Soc.* 73, 3923.
13. CLARKE, JOHNSON and SIR R. ROBINSON, 1949, *The Chemistry of Penicillin* Princeton University Press Princeton, 498, 525, 642.

The Mechanism of the Vapour Phase Nitration of Butane

Nitroparaffins are produced on industrial scale by vapour nitration of gaseous paraffins with nitric acid, but little is known on the mechanism of the reaction. Only recently (after the present investigation had been completed), some problems involved in the process have been elucidated by Bachman and co-workers[1].

In order to obtain an insight into the mechanism of vapour phase nitration, a number of factors have been studied which were expected to affect the course of the nitration of butane (used in form of buta-gas).

1) A number of experiments were carried out, in which the *ratio R* paraffin: nitric acid was changed systematically. Figure 1, in which the conversion is plotted versus the ratio R, shows $R=3$ to be the optimum ratio. Figure 2, which illustrates the *quantity* of nitroparaffins formed per minute as function of the ratio R, on the other hand, indicates that the quantity of nitroparaffin increases with decreasing R. As a ratio smaller than 2 would bring the mixture near to the limits of explosibility, the following experiments were carried out with a ratio R of 2.3.

2) The influence of the *contact time* was determined by changing the reactor volume whilst keeping the rate of flow of the reactants constant. Statistical evaluation of these experiments led to an optimum contact time of 3.8—4.1 sec.
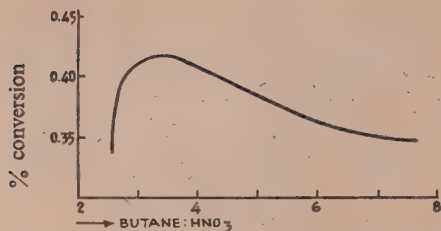
Figure 1

Nitration of butane. — Flow rate of butane: 60—80 mM/min. Temp.: 430—440°C.
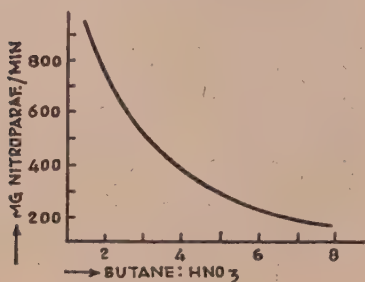
Figure 2.

Nitration of butane. — Flow rate of butane: 60—80 mM/min
Temp.: 430—440°C.
Contact time: 0.517—0.915 sec.
Pressure: 1140—1375 mm Hg.

3) The surprising observation was made that not only the volume of the reactor (i.e. the contact time) is an important factor, but also the *geometry of the reactor*, i.e. the ratio surface/volume: the conversion increases when this ratio increases.

This was the first indication that the reaction is not homogeneous.

4) For each reactor, the *optimum temperature* was determined, by plotting the quantity of organic layer in the reaction product (also an aqueous layer containing, *inter alia*, various oxidation products of the butane, is formed) *versus* the temperature. Figure 3 gives two examples of the curves obtained; the optimum temperature lies obviously in the neighbourhood of 380°.

5) It has been known that vapour phase nitration of a paraffin gives not only the corresponding nitroparaffin, but the nitro-derivatives of all lower paraffins down to nitromethane, as well. In Table I, the results of the fractionation analysis of the nitroparaffins obtained in a number of cases from butane are summarised. It appears that the ratio surface/volume has an influence on the relative quantities of *nitromethane* found in the reaction product. This "cracking reaction"
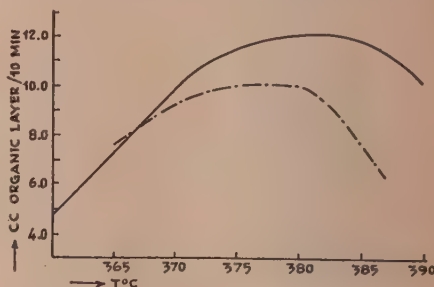
Figure 3

Nitration of butane. — Flow rate of butane: 80 mM/min
Ratio butane: HNO₃ 2.3 — 2.5.
—.—. Reactor 580 cc; 16 mm diameter.
———— Reactor 238 cc; 16 mm diameter.

TABLE I

Nitration of Butane

Effect of surface: Volume ratio on the concentration of the various nitroparaffins in the product.

Experiment No.	B 121	B 156	B 178	B 193	B 91	B 199	B 201
1. Reactor Volume, cc	238.0	238.0	238.0	238.0	238.0	238.0	238.0
2. Reactor Diameter, mm	43	16	43—16	43—16	5	43, with 30 cc Raschig rings	43, with 50 cc Raschig rings
3. Ratio of Surface to Volume (cm-1)	1.00	2.50	1.55	1.55	7.98	4.47	6.53
4. Ratio of Heated Surface to Contact Surface	1	1	0.73	1	1	0.35	0.186
5. Temperature, °C	390	380	388	375	429	375	370
6. Butane mM/min	80.8	80.0	80.0	80.0	83.2	80.0	80.0
7. Ratio Butane/HNO₃	2.47	2.31	2.30	2.29	2.55	2.29	2.26
8. Contact time, sec.	1.70	1.63	1.64	1.61	1.65	1.56	1.56
9. Nitromethane, weight-%	8.2	4.2	5.4	17.7	7.2	25.2	17.5
10. Nitroethane, weight-%	7.2	22.0	22.1	19.3	14.1	31.4	26.7
11. Nitropropanes, weight-%	44.3	31.6	29.8	56.2	30.2	19.9	33.9
12. Nitrobutanes, weight-%	40.3	42.2	42.7	6.8	48.5	23.5	21.9
13. Conversion of HNO₃, %	25.3	32.6	31.6	32.1	34.9	30.7	33.3

undoubtedly points to the conclusion that the vapour phase nitration of paraffins involves free radicals.

This explanation makes the importance of the ratio surface/volume understandable. Increase of the surface will favour short reaction chains and minimise secondary reactions in favour of the following one:

$$HNO_3 \rightarrow \cdot NO_2 + \cdot OH$$
$$\cdot OH + CH_3R \rightarrow H_2O + \cdot CH_2R$$
$$R.CH_2 \cdot + \cdot NO_2 \rightarrow R.CH_2.NO_2$$
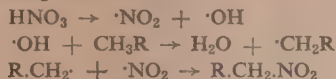
Secondary reactions lead to the various oxidation products observed in the reaction (see above).

Evidence in support of this mechanism can be seen in the observation that NO, a known inhibitor of radical reactions, is detrimental to the efficiency of the vapour phase nitration. Addition of 5% of NO to the butane leads to zero conversion.

By plotting the logarithm of the contact time t_c *versus* 1/T, a straight line is obtained for all reactors of the same diameter. Reactors of different diameter give different straight lines — but their slopes are identical (see Figure 4). This slope
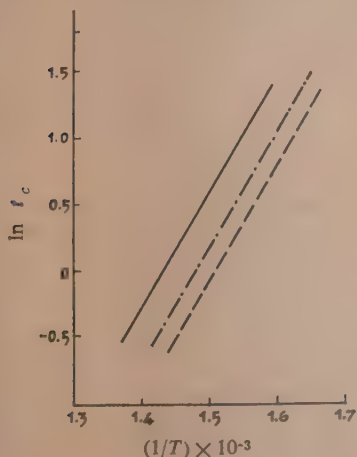
Figure 4
Nitration of butane. ($t_c = e^{-Eact./R-T}$)—Flow rate of butane: 80 mM/min.
Ratio butane: HNO₃   2.3 — 2.5.
———— Reactor 5 mm diameter.
— — — Reactor 16 mm diameter.
—.—.— Reactor 43 mm diameter.

gives the activation energy of the vapour phase nitration of butane as — 35.5 kcal/mole.

A. CANTONI
A. ROSENZWEIG
J. LEONZINI
S. BERKOVITS
Scientific Department,
Ministry of Defence

REFERENCE
1. BACHMAN *et al.*, 1952, *J. Org. Chem.*, 17, 906.

The Isotopic Exchange of Oxygen and Hydrogen between Water and Alcohols

For a large number of organic reactions, especially those involving alcohols in aqueous medium such as esterification, dehydration, etc., a mechanism was suggested whereby a positive carbonium ion appeared as intermediate product.

In order to investigate this assumption, the exchange reaction of oxygen between an alcohol and water was chosen, representing one of the simplest substitution reactions at a saturated carbon atom. This reaction can take place by one of two mechanisms:

1. Elimination of, and recombination with, a water molecule, with the formation of an olefine

$$H - C - COH \rightleftharpoons C = C + H_2O$$
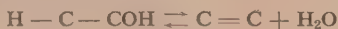
2. Fission of the carbon-oxygen bond with the formation of a carbonium ion

$$C - C - OH \rightleftharpoons C - C^+ + OH^-$$

which by a further step can also form an olefine. A very convenient method for the investigation of the mechanism of this reaction is by means of using O¹⁸- labelled compounds. In our experiments the oxygen of either alcohol or water was labelled with O¹⁸ and the condition and course of the reaction followed by means of ·isotopic-analysis of the reaction products.

Methods were developed for the preparation of labelled alcohols, separation and purification of the products and a method of mass-spectometric analysis of alcohols was devised.

The rates of exchange of primary, secondary and tertiary alcohols have been compared. As expected, the rate of exchange increases rapidly in going from primary to tertiary alcohols. For example, the rate of exchange of *t*-butyl alcohol is some 3000 times that of *sec*-butyl alcohol under similar conditions. This factor is of the same order of magnitude as that observed in the unimolecular hydrolysis of alkyl halides.

The exchange reaction is catalysed by hydrogen ions, and the rate is independent of the nature of the acid. No exchange has been observed in alkaline and neutral solution.

From measurements at a number of temperatures, Arrhenius parameters for these reactions have been calculated. For example, the Arrhenius activation energy for the exchange of *t*-butyl alcohol in 0.1 N sulphuric acid is 31 kcal/mole.

I. DOSTROVSKY
F. KLEIN
D. SAMUEL
Department of Isotope Research,
Weizmann Institute of Science,
Rehovot

Hydrolysis of Tertiary Butyl Hypochlorite

In the course of our investigation of the mechanisms of reaction of organic hypohalites. we have investigated the hydrolysis of tertiary butyl hypochlorite.

Hydrolyses were carried out of normal tertiary butyl hypochlorite in heavy-oxygen-enriched water and of heavy tertiary butyl hypochlorite in normal water. Isotopic analysis of the products of the hydrolyses shows that in alkaline and acid media it is always the bond between the oxygen and the chlorine which is broken in the course of the reaction.

Similar experiments involving the formation of tertiary butyl hypochlorite from tertiary butanol (both normal and heavy), water (both normal and heavy), and chlorine have shown similarly, that it is the bond between oxygen and the hydrogen of the hydroxyl group which is broken in the course of reaction.

Kinetic investigation was carried out of hydrolysis and formation reactions of tertiary butyl hypochlorite under various conditions of acidity, alkalinity and acidity, temperature, salt concentration and solvents. From these experiments it is possible to obtain information regarding the mechanism of these reactions as well as to derive certain thermodynamical quantities. The equilibrium constant of this reaction was obtained both by direct measurements and also by calculation from the kinetic data.

M. ANBAR
I. DOSTROVSKY
Department of Isotope Research,
Weizmann Institute of Science,
Rehovot

The Relationship between Ionisation and Rearrangement of Aliphatic Compounds

A large number of aliphatic compounds undergo rearrangement of the carbon skeleton on undergoing chemical reactions.

It has been shown that such rearrangements of the Wagner-Meerwein or of the pinacol types occur only when the mechanism of the reaction is "unimolecular", i.e., a two-stage mechanism in which the first step involves heterolytic fission of a bond C-X extending from a carbon atom with the formation of an intermediate carbonium ion.

Nevertheless the precise role of the rearranging group and the sequence of events in the course of the reaction was not clear, and certain apparently conflicting results have been reported.

This work was undertaken in order to determine whether rearrangement is simultaneous with, or subsequent to, the first ionisation stage. Evidence was obtained from kinetic measurements on the rate of fission of the C-X bond in a number of aliphatic bromides in media of varying ionising power and nucleophilic power. The effect of alkyl and aryl groups on the rate of solvolysis was also determined. Attempts were made to obtain additional evidence as to the participation of the rearranging group by relating the stereochemistry of the starting material and the products of rearrangement. It was found that the phenyl group in an α-position to the C-X bond exerts a stabilising influence even in a neopentyl system.

In the case of the rearrangement of a methyl group, the rearrangement is subsequent to, and independent of, the ionisation, in distinction to cases where an aryl group rearranges. The difference between these two cases can be explained theoretically.

I. DOSTROVSKY
DAVID SAMUEL
Department of Isotope Research
Weizmann Institute of Science,
Rehovot

A Modified Peptide Synthesis

In a recent investigation on the action of dry hydrogen bromide or of hydrogen chloride on benzyl carbamates, it was observed that hydrogen bromide in glacial acetic acid is an excellent reagent for the cleavage of carbobenzoxy groups attached to nitrogen. In the case of benzyl esters of N-carbobenzoxy-α-amino acids the carbobenzoxy groups are preferentially cleaved leading to benzyl ester hydrobromides of α-amino acids.

A modified peptide synthesis, based on the above observations. is suggested:

$$\underset{I}{C_7H_7OCONHCHCOCl} + HBr. \underset{II}{NH_2\overset{R_1}{C}HCOOC_7H_7} \xrightarrow{KHCO_3}$$

$$\underset{III}{C_7H_7OCON H \overset{R}{C}HCONH\overset{R_1}{C}HCOOC_7H_7}$$

III

$$HBr \Big\downarrow CH_3COOH \qquad\qquad Pd-C \atop H_2 \Big\downarrow$$

$$\overset{R}{\underset{|}{}}\quad\overset{R_1}{\underset{|}{}}$$

$$CO_2 + C_7H_7Br + HBr.\ NH_2CHCONHCHCOOC_7H_7 \qquad\qquad NH_2CHCONHCHCOOH + 2C_7H_8 + CO_2$$

IV V

$$\overset{R}{\underset{|}{}}\quad\overset{R_1}{\underset{|}{}}\quad\overset{R_2}{\underset{|}{}}$$

$$I + IV \xrightarrow{KHCO_3} C_7H_7OCONHCHCONHCHCONHCHCOOC_7H_7$$

VI

$$H_2 \downarrow Pd-C$$

$$\overset{R}{\underset{|}{}}\quad\overset{R_1}{\underset{|}{}}\quad\overset{R_2}{\underset{|}{}}$$

$$NH_2CHCONHCHCONHCHCOOH + 2C_7H_8 + CO_2$$

VII
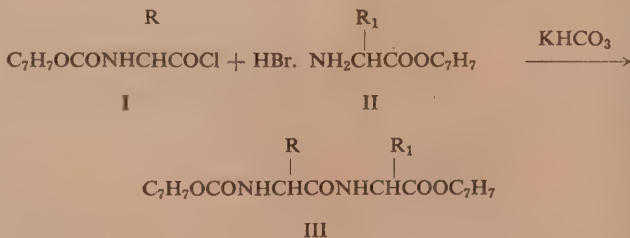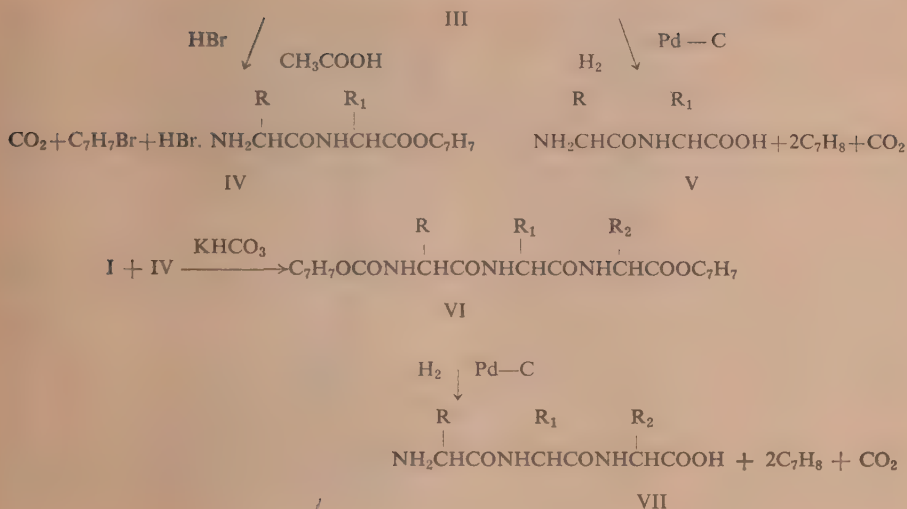
The N-carbobenzoxy-α-amino acid chlorides (I) are condensed with the benzyl ester hydrobromide of the α-amino acid (II) or the peptide benzyl ester hydrobromide (IV) in a mixture of ethyl acetate and water in the presence of potassium bicarbonate (Schotten-Baumann).

Hydrogen bromide in glacial acetic acid (33%) cleaves the carbobenzoxy group of the N-carbobenzoxy peptide benzyl ester and yields the corresponding peptide benzyl ester hydrobromide (IV).

Catalytic hydrogenation of the benzyl ester of the N-carbobenzoxy peptide (III, VI) in methanol over Pd-C cleaves both the carbobenzoxy and the benzyl groups and leads directly to the free peptide (V, VII).

DOV BEN-ISHAI
*Weizmann Institute of Science,
Rehovot*

On an Azulene and a New Azulenogenic Sesquiterpene from Artemisia Arborescens L.

Steam distillation of *Artemisia arborescens* L., which is fairly frequent in Israel, resulted in a blue essential oil (yield 0.9%), containing an azulene whose analysis is consistent with the formula $C_{15}H_{18}$. This azulene was identified as chamazulene (the azulene from camomile) by means of its derivatives, its absorption spectrum, and other physical data.

It was also possible to isolate from the cold extract of *Artemisia Arborescens* a hitherto unknown sesquiterpene (yield 0.2%) which corresponded to the formula $C_{15}H_{20}O_3$. This substance yields chamazulene, upon dehydrogenation with selenium. Reactions and degradation products of the compound are described which prove that it contains a double bond and, as functional

groups, a lactonic and an ether group, and which also give information on the relative positions of these substituents.

A. MEISELS
ANNA WEIZMANN
*Weizmann Institute of Science,
Rehovot.*

Enolates of α-Fluorocarboxylic Acid Esters and their Properties

The C-F bond is much more resistant to hydrolysis, especially under alkaline conditions, than all other C-halogen bonds. It seemed, therefore, worthwhile investigating whether the enolates of α-fluoro-esters would be sufficiently stable to permit the condensation reactions characteristic of non-halogenated esters. A positive answer to this question would make it possible to carry out syntheses of complicated fluorine containing compounds, some of which would be of considerable biological interest.

To some extent, such a positive answer could be anticipated from an extrapolation of the properties of analogous derivatives of the other halogens. Whilst, e.g., ethyl α, γ-dichloro-acetoacetate can be prepared from ethyl chloroacetate and (alcohol-free) sodium ethoxide[1], ethyl bromoacetate is not capable of a similar self-condensation, and whilst diethyl chloromalonate can be benzylated in the usual manner[2], one obtains from diethyl bromomalonate only tetraethyl ethylenetetracarboxylate under these conditions[3]. Both diethyl chloro- and bromomalonate react normally with the enolate of ethyl acetoacetate[4].

Indeed, the following observations show that α-fluoro-esters are capable of the normal reactions of enolates.

1) *Diethyl oxalo-fluoroacetate*, $H_5C_2OOC.CO.CHF.COOC_2H_5$. The condensation of diethyl oxalate and ethyl fluoroacetate with alcohol-free sodium ethoxide in anhydrous ether gives a sodium derivative (enolate) which is soluble in water. The parent substance is isolated by acidification of the aqueous solution and extraction with ether. Thus, diethyl oxalo-fluoroacetate is obtained as a colourless oil, b.p. 120—122°/9 mm; $d_{20}^{20} = 1.420$. The yield is 25—30%. The substance gives the typical ferric chloride colour reaction and is characterised by a 2,4-dinitrophenylhydrazone of m.p. 122° (from alcohol). The infrared spectrum (0.021 g and 1 ml of carbon tetrachloride) was measured in a cell of 0.5 mm thickness, and the following absorption bands were observed: a broad, very strong band from 1738—1750 cm-1, representing the combined carbonyl absorption of the keto and ester groups, a band at 1094 cm-1 (optical density $d = 1.1$) which is assigned to the C-F bond, and two bands at 1440 cm-1 ($d = 0.040$) and 1368 cm-1 ($d = 0.60$), respectively, which belong to the methyl radicals in the ethyl groups. The C-F absorption has been located at 1045 cm-1 in fluoroacetic acid; it was to be expected that the neighbourhood of the carbonyl double bonds would raise the wave number of the C-F band. For analogous reasons, the carbonyl frequency is unusually high[5]; a similar effect of *bromine* atoms has been observed by Jones *et al.*[6].

2) *Ethyl α, γ-difluoro-acetoacetate*, $CH_2F.CO.CHF.COOC_2H_5$. Self-condensation of ethyl fluoroacetate under the conditions described above, yielded the desired ester in satisfactory yield, as a colourless liquid of b.p. 120—130°/20 mm; $d_{20}^{20} = 1.415$. The compound shows the same ferric chloride reaction as ethyl acetoacetate and gives a 2,4-dinitrophenylhydrazone of m.p. 125—126° (from alcohol). The infrared spectrum (0.021 g and 1 ml of carbon tetrachloride; cell thickness 0.5 mm) is very similar to that of ethyl oxalo-fluoroacetate: very strong carbonyl band at 1752—1762 cm-1 ($d = 1.4$); strong C-F band at 1100 cm-1; methyl absorption at 1440 cm-1 ($d = 0.35$) and 1373 cm-1 ($d = 0.45$).

3) Ethyl α, γ-difluoro-acetoacetate can be *benzylated* by the classical method (treatment with sodium ethoxide in alcohol and benzyl chloride). It also undergoes the *Michael condensation* with diethyl maleate according to the following equation, leading to triethyl 1,3-difluoro-2-keto-pentane-3, 4, 5-tricarboxylate:

$H_5C_2OOC-CH$
$\qquad \|\qquad + CH_2F.CO.CHF.COOC_2H_5 \rightarrow$
$H_5C_2OOC-CH$

$H_5C_2OOC-CH-CF.COOC_2H_5$
$\qquad\quad |\qquad |$
$H_5C_2OOC-CH_2CO.CH_2F$
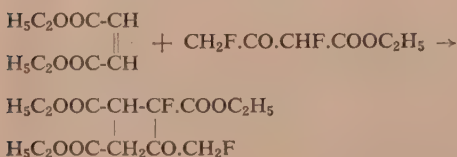
4) Diethyl fluoromalonate, prepared from diethyl chloromalonate and potassium fluoride[7], can also be *benzylated* to diethyl fluoro-benzyl-malonate and *adds to diethyl maleate* in presence of basic catalysts to give tetraethyl 1-fluoropropane-1,1,2,3-tetracarboxylate.

We are indebted for the infra-red spectra to Dr. S. Pinchas, Weizmann Institute of Science.

I. BLANK
E. D. BERGMANN
Scientific Department
Ministry of Defence
J. MAGER
Israeli Institute of Biological
Research.

REFERENCES

1. WISLICENUS, 1910, *Ber.*, 43, 3532.
2. CONRAD, 1881, *Ann.*, 209, 243.
3. FINKELSTEIN, 1910, *Ber.*, 43, 1532.
4. GAULT and KLEE, 1926, *Bull. Soc. Chim. France*, (4), 39, 1009.
5. BERGMANN E. and PINCHAS, 1952, *J. Chim. physique*, 49, 537.
6. JONES *et al.*, 1952, *J. Amer. Chem. Soc.*, 74, 2828.
7. BLANK (private communication).

The Vulcanisation of Rubber Latex in Solution

In connection with a study of solutions of high-molecular compounds in low boiling non-polar solvents, the problem posed itself whether rubber could be vulcanised without losing its solubility, so as to give stable gels of high tensile strength. As rubber vulcanised in bulk even at low temperature proved insoluble in paraffin hydrocarbons, vulcanisation *in solution* and at low temperature (25°C) was studied, using sulphur monochloride (S_2Cl_2)[1] as vulcanising agent. The rubber solutions used had a concentration of 2—5%, and the sulphur compound was employed in quantities of 1.2—1.5% of the rubber. If one plots the tensile strength of the solution as a function of time, one observes that 4—12 hours after the vulcanisation, there appears for a short period the desired tensile strength and viscoelasticity which, however, disappear normally again in the course of time. The length of this short period depends on external factors such as the rate at which the reactants are mixed, the temperature, the previous history of the rubber latex, etc.

The problem was, therefore, to interrupt these further reactions and to stabilise the solutions in the state in which they show the desired properties. It could be shown that the change in properties which was to be avoided, is due to three main factors: autoxidation, the acid formed in the presence of vulcanisation, and traces of water. Inorganic bases which were investigated, had no influence, organic bases (pyridine, diphenylamine, aniline, diphenylguanidine) brought about a definite improvement, but on the whole did not give the solution both stability and the desired tensile length.

A type of stabilisers which is being industrially, are soaps, e.g., zinc stearate, which, however, are not soluble in paraffinic solvents. It was found that the soaps of tervalent metals, e.g. aluminium distearate, are both soluble in paraffins and give together with organic bases, e.g., diphenylamine, the vulcanised rubber latex solutions the required properties. Both the aluminium soap and the diphenylamine were employed in quantities of 0.2—0.3 % of the rubber.

In order to improve the method still further, vulcanising agents are being investigated which will not produce acid as by-product. Preliminary experiments have shown that the so-called alkyl thiosulfites ROS.SOR which are prepared from sulfur monochloride and alkali alkoxides, can be employed to advantage in the above procedure.

The theory of the vulcanisation process in solution is as obscure as that of vulcanisation in general. For the vulcanisation of an olefinic unit in rubber, at ordinary or elevated temperature, one assumes an asymmetric type of reaction, thus:

$$2-CH_2-CH=C-CH_2- \rightarrow -CH_2-CH-C-CH_2$$

(A)
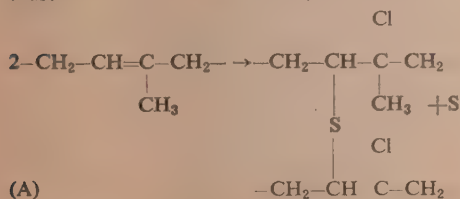
but at low temperatures the formation of disulfide bonds has also been postulated [2,3,4,5]:

$$2-CH_2-CH=C-CH_2- \rightarrow -CH_2-CH-C-CH_2$$

(B)
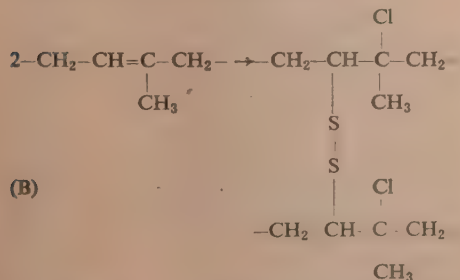
In both cases, the compounds formed can slowly split off hydrogen chloride (apart from the hydrogen chloride liberated initially), which would cause gradual changes in the molecular structure of the vulcanised gel. Furthermore, the finely divided sulfur formed in reaction (A) can also cause secondary reactions.

A third, intramolecular type of reaction has been postulated[6] as occurring in the interaction of sulphur monochloride and a rubber molecule:

$$- CH_2-CH-C-CH_2 --- CH_2-CH=C-CH_2 - \rightarrow$$

(C)

$$-CH_2-CH-C-CH_2 -- CH_2-CH-C-CH_2- +S$$
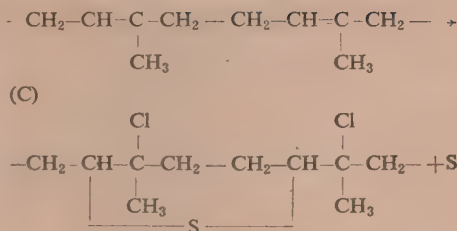
Hydrogen chloride, split off from such a molecule, would be able to open the heterocyclic ring and thus create points at which secondary reactions, e.g. cross-linking would occur. The combination of aluminium distearate and diphenylamine appears to prevent secondary reactions under all circumstances.

SH. BEN-MOSHE
A. OBLATH
E. UZIEL
Scientific Department,
Ministry of Defence

REFERENCES

1. WEBER, Ztsch. Angew. Chem., 1894, 7, 112,142,; WEBER, 1902, The Chemistry of Indian Rubber, (Griffin and Co., London), 104.
2. POPE, 1920, J. Chem. Soc., 117, 271; 1921, 119, 396; 1922, 121, 1166.
3. MEYER and MARK, 1928, Ber., 61, 1948.
4. KEMP and SELKER, 1944, Ind. Eng. Chem., 36, 20.
5. HULL, OLSEN and FRANCE, 1946, Ind. Eng. Chem., 38, 1273.
6. MIDGLEY, HENNE and SHEPARD, 1932, J. Amer. Chem Soc., 54, 2153.

Homo-hypoxanthine

The elucidation of the central role of the purines (e.g., I, hypoxanthine) in biological processes has led to the synthesis and study of "antimetabolites" of analogous structure, e.g., the triazolo-pyrimidines (II, 8-azahypoxanthine)[1,2,3]. In the present investigation, the corresponding homo-

I   II

III
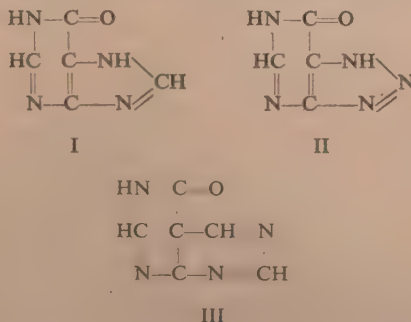
hypoxanthine[4]. (III) was prepared. The synthesis is illustrated by the following chart:

$$CH_3-C-CH=N$$
$$\underset{HC-N=CH}{\overset{\|}{}}\quad\xrightarrow{HNO_3}$$

IV

$$N=CH-C-CH=CH-C-CH=N$$
$$\underset{CH=N-C-NO\quad ON-C-N=CH}{\overset{\|}{}}\quad\xrightarrow{KMnO_4}$$

V

$$HOOC-C-CH=N$$
$$\underset{O_2N-C-N=CH}{\overset{\|}{}}\quad\xrightarrow{NaS_2O_4}$$

VI

$$HOOC$$
$$\underset{H_2N-C-N=CH}{\overset{\overset{|}{C}-CH=N}{\overset{\|}{}}}\quad\xrightarrow[HCONH_2]{HCOOH}\quad III$$

VII
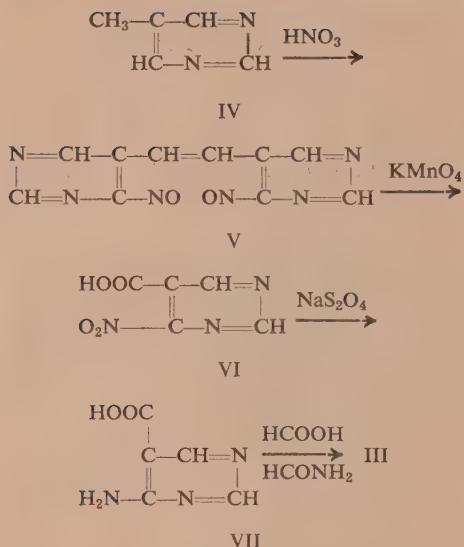
4-Methyl-pyrimidine (IV)[5] was subjected to nitro-oxidation and gave a neutral product of m.p. 131—132°, which according to the analysis had formula (V) of a di-(5-nitroso-pyrimidyl-4)-ethylene (*Anal.* Calcd. for $C_{10}H_6N_6O_2$: C, 48.8; H, 2.5; N, 34.7. Found: C, 49.0; H, 2.5; N, 34.6%). The "dimerisation" of the molecule is undoubtedly due to the reactivity of the methyl group in (IV) which resembles that of 2- or 4-methyl-pyridine[6]. (V), upon oxidation with potassium permanganate, gave in 20% yield 5-nitro-pyrimidine-4-carboxylic acid (VI) of m.p. 236—237° (dec.), which was smoothly reduced by sodium hydrosulfite to the corresponding amino-acid (VII), yellow needles, of m.p. 232° (dec.) (from alcohol). The synthesis was completed by heating (VII) at 180—200° with a mixture of formic acid and formamide[7]. Homo-hypoxanthine (III) formed, after recrystallisation from ethyl alcohol, a colourless crystalline powder which melted at 317° dec.

The biological properties of (III) are now being investigated.

E. ESCHINAZI
E. D. BERGMANN
Scientific Department,
Ministry of Defence

REFERENCES

1. KIDDER and DEWEY, 1949, *J. Biol. Chem.*, **179**, 181.
2. KIDDER, DEWEY, PARKS and HEINRICH, 1950. *Proc. Natl. Acad. Sci. U. S.*, **36**, 431.
3. KIDDER, DEWEY and PARKS, 1951, *Fed. Proc.*, **10**, 207.
4. For the nomenclature, see FISCHER and ROCH, 1951, *Ann.*, **572**, 217.
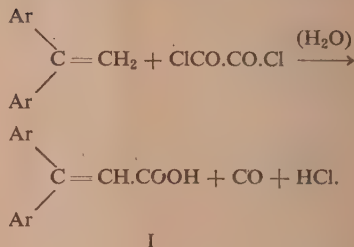5. GABRIEL and COLMAN, 1899, *Ber.*, **32**, 1534.
6. See, e.g., GABRIEL and COLMAN, 1903, *Ber.*, **36**, 3383.
7. FISCHER and ROCH, *l. c.*

The Reaction of 1,1-Diarylethylenes with Acid Chlorides

1) The reaction of 1,1-diarylethylenes with oxalyl chloride according to Kharasch gives acrylic acids (I), with liberation of hydrogen chloride and carbon monoxide:

$$\underset{Ar}{\overset{Ar}{>}}C=CH_2 + ClCO.CO.Cl \xrightarrow{(H_2O)}$$

$$\underset{Ar}{\overset{Ar}{>}}C=CH.COOH + CO + HCl.$$

I

In experiments with 1,1-di-(*p*-chlorophenyl)-ethylene, there was isolated as by-product in minor quantities an α-keto-acid (II), m.p. 134—135°, which represents the first step of the reaction. Its structure was proven by condensation of the same olefine with oxalester chloride:

$$(Cl.C_6H_4)_2\ C=CH_2 + ClCO.COOC_2H_5 \longrightarrow$$
$$(Cl.C_6H_4)_2\ C=CH.CO.COOC_2H_5$$

IIa

$$\longrightarrow (Cl.C_6H_4)_2.C=CH.CO.COOH.$$

II
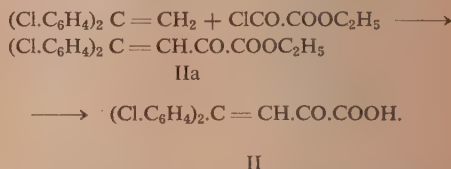
(IIa) has m.p. 93—94°; dinitrophenylhydrazone, m.p. 202—205°; U.V. spectrum: λ_{max} 2320; 3170 A (log ε = 4.21; 4.18). This is a general method for the preparation of unsaturated α-keto acids of the type (II). The halogen-free analog of (IIa) melts at 49—50°; dinitrophenylhydrazone, m.p. 180—184°: U.V. spectrum: λ_{max} = 2300; 3140 A (log ε = 4.02; 4.12). The halogen-free acid corresponding to (II) has m.p. 125°.

2) A similar activating influence on the COCl radical has also been observed in the case of trichloro- and dichloro-acetyl chloride which yield with 1,1-diphenylethylene the ketones (III) and (IV):

$$(C_6H_5)_2\ C=CH_2 + CCl_3.COCl \longrightarrow$$
$$(C_6H_5)_2\ C=CH.CO.CCl_3 \qquad\text{(III)}$$

$$(C_6H_5)_2\ C=CH_2 + CHCl_2.COCl \longrightarrow$$
$$(C_6H_5)_2\ C=CH.CO.CHCl_2 \qquad\text{(IV)}$$
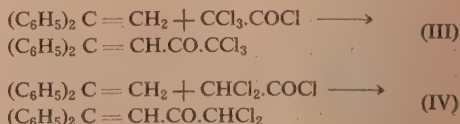
(III) melts at 84°, (IV) is an oil of b.p. 175—180°/3 mm. (IV) forms with 2,4-dinitrophenylhydrazine a hydrazone of m.p. 164—168° and an osazone ob m.p. 248°.

3) The reaction between aryl-olefines and aromatic acid chlorides which has been reported on in the previous Convention, is applicable also to styrene and its derivatives. In the condensation with phenylacetyl chloride, styrene yields an enol ester of type (VI), m.p. 138°, exclusively in the *trans-trans*-form, and a mixture of isomeric ketones (V), from which a crystalline ketone of m.p. 70—71° could be isolated. The enol-ester (VI) has been characterised by its adduct with maleic anhydride, m.p. 155°.

Analogously, from α-methylstyrene, one obtains 2,5-diphenyl-2-penten-4-one (VII), b.p. 145°/4 mm, characterised by a dinitrophenylhydrazone of m.p. 123°, and an enol ester (VIII), m.p. 104°, and from 1-phenylbutadiene 1,6-diphenyl-1,3-hexadien-5-one, m.p. 101° (IX), dinitrophenylhydrazone, m.p. 184°, together with the enol ester (X), m.p. 130°.

In the case of phenylacetylene and phenylacetyl-chloride, it has proven impossible to hydrolyse the enol-ester (XI), (m.p. 65°) obtained to the ketone; it is necessary for the synthesis of the ketone, to apply Nef's method.

$$C_6H_5.CH = CH_2 + ClCOCH_2.C_6H_5 \longrightarrow$$
$$C_6H_5.CH = CH.CO.CH_2C_6H_5 \quad \quad \quad (V)$$

$$C_6H_5.CH = CH.C = CH.C_6H_5 \quad \quad (VI)$$
$$| \quad\quad\quad\quad\quad\quad OCOCH_2.C_6H_5$$

$$C_6H_5.C(CH_3) = CH.CO.CH_2.C_6H_5 \quad (VII)$$

$$C_6H_5.C(CH_3) = CH.C = CH.C_6H_5 \quad (VIII)$$
$$OCOCH_2.C_6H_5$$

$$C_6H_5.CH = CH.CH = CH.CO.CH_2.C_6H_5 \quad (IX)$$

$$C_6H_5.CH = CH.CH = CH.C = CH.C_6H_5 \quad (X)$$
$$| \quad\quad\quad\quad\quad\quad OCOCH_2.C_6H_5$$

$$C_6H_5C = C.C = CH.C_6H_5 \quad (XI)$$
$$| \quad\quad\quad OCO.CH_2C_6H_5$$
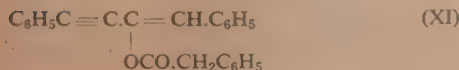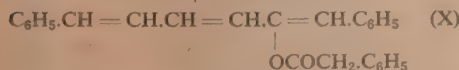
F. BERGMANN
J. KLEIN
Department of Pharmacology,
Hebrew University-
Hadassah Medical School,
Jerusalem

S. SAR-EL
Department of Organic Chemistry,
Hebrew University

A. KALMUS
Scientific Department,
Ministry of Defence

The Influence of Physical and Chemical Factors on the Solid Phase Reaction Between Potassium Perchlorate and Carbon

Most solid-solid reactions are very slow, and only a small part of the reactants enters into reaction, due to a mechanical interference of the reaction products[1]. The oxidation of carbon by potassium perchlorate, a true sol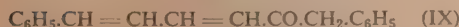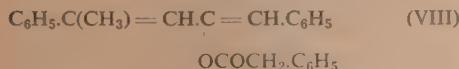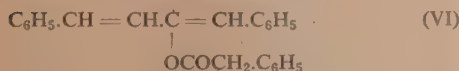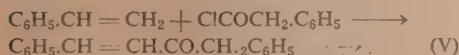id-solid reaction which goes practically to completion in relatively short time[2], affords a useful model for this type of process because of the volatility of one of the reaction products (carbon dioxide) and the great mobility of potassium chloride in the crystal lattice.

The evolution of carbon dioxide makes accurate kinetic measurements possible. In the present investigation, the influence of a number of physical and chemical factors on the course of the reaction has been studied, such as: particle size, method of mixing the reactants, which were investigated in the form of tablets; the pressure used in making the tablets; pressure in the reaction chamber; catalytic influence of addition of inorganic salts.

The kinetic study in the case of the systems potassium perchlorate - soot and potassium perchlorate - Nuchar (used in equivalent quantities) has shown that decrease in size of the perchlorate particles leads to an increase in reaction velocity. However, it has not yet been possible to find a quantitative relationship between particle size and the rate at which carbon dioxide is evolved.

In order to obtain reproducible results, it is necessary to use always the identical procedure in mixing the reactants and preparing the tablet. An important factor is the wetting of the material before the compression — the humidity gives the tablets the required mechanical stability and permits preservation of their form during the course of the experiment. Tablets prepared in this manner, gave reproducible results.

Preliminary kinetics measurements, carried out under varying nitrogen pressure (60—80 atmospheres) did not reveal any significant influence of the pressure on the reaction rate.

In further experiments, the catalytic influence of inorganic catalysts on the potassium perchlorate oxidation of soot, Nuchar and graphite has been studied. Previous investigations[3] have shown that inorganic salts have a very pronounced catalytic influence on the oxidation of soot by the oxygen of the air, basic salts and alkali halides representing two effective groups of catalysts. In the oxidation of carbon by potassium perchlorate, only the halides of the alkali metals and of the alkaline earths act catalytically, not, however, the basic salts. It has to be concluded that in this oxidation by potassium perchlorate, surface oxides of the carbon do not function as intermediates; it is more than probable that the

reaction between the two components of the mixture takes place in a single step.

E. HOFFMANN
J. RAJBENBACH
S. PATAI
Scientific Department,
Ministry of Defence

1. For a review of reactions in the solid phase, see COHEN, 1928, *Chem. Rev.*, **42**, 527.
2. PATAI and HOFFMANN, 1950, *J. Amer. Chem. Soc.*, **72**, 5098.
3. PATAI, HOFFMANN and RAJBENACH, 1952, *J. Applied Chem.*, **2**, 306; PATAI, RAJBENACH and HOFFMANN, 1952, *ibid.*, **2**, 311.

Papers Presented in the Section of Inorganic and Physical Chemistry

Problems of Laboratory Research on Artificial Rain

1. The cloud as a physical system (water aerosol), its formation and physical properties. The mechanism of rain formation.

Nucleation of liquid, supercooled and supersaturated aerosols.

Explanation of the processes of condensation, crystallisation and nucleation on the basis of the theory of phase transition.

2. The deep freezer as cloud chamber.

Transformation of supercooled water drops to ice by seeding with solid carbon dioxide and silver iodide smoke.

Production of silver iodide smoke, determination of its concentration and of the size of its particles (electron microscope); growing of the smoke particles on supersaturated droplets.

"Threshold temperature" of the seeding.

Study of the ice crystals formed by nucleation (Schaeffer's replica method).

3. Investigation of other compounds (cadmium iodide, lead iodide) as nucleators.

Explanation of nucleating activity in terms of similarity in crystal structure of nucleator and ice.

Extension of the method of growing nucleator crystals on droplets supersaturated with the same substance, to the use of droplets supersaturated with a different compound but of similar structure.

Improvements in the methods of generation of aerosols.

4. Outstanding problems:
(a) Standardisation of experimental conditions (clouds of constant particle concentration and size; nucleating smoke of constant particle concentration and size; temperature constancy, etc.).
(b) Study of the efficiency of nucleation of (non-aqueous) aerosols by crystals of similar lattice structure. The relationship between efficiency and crystal structure.

M. D. BITRON
E .RAPAPORT
Tel Aviv

An Extension of the Notion of Brisance and its Absolute Measure

The brisance of an explosive is practically defined as "its ability to shatter objects in the immediate vicinity of the explosion". It is usually measured by methods based either on the permanent deformation of plastic materials (Hess: lead cylinders; Kast: copper cylinders; Trauzl: cavities in lead blocks), on the fragmentation of brittle bodies (American sand test; fragmentation of a steel cylinder), or on the power of the exploding substance to initiate other explosives (Bollé)[1]. These methods are, however, not related at all to the more exact definitions of brisance[2] such as the following two which will be shown to be fundamentally identical.

One of them, put forward by Kast and by Woehler (Brisance $B = \frac{E}{t} d$, where d=density of the explosive, t=time, E=energy of the explosion), considers brisance as a power developed in a given volume (dimension: (ml^2t^{-3}) (l^{-3}) = $(ml^{-1}t^{-3})$; practical unit: one kilowatt per litre= one watt per cc or 10^7 units g.c.s.). The second, going back to Vieille, Sarraut and Petavel, takes brisance as a time-pressure gradient (dimension: $(mlt^{-2})(l^{-2})(t^{-1})$=$(ml^{-1}t^{-3})$ as above; practical unit: one atmosphere per second=1.01325×10^6 units g.c.s.). The passage from one practical unit to the other will be as follows:

1 kilowatt per litre=9.869 atmosphere per second, 1 atmosphere per second=0.101325 kilowatts per litre.

According to the first definition, the work done by an explosive decomposing within a finite, constant volume v is (for a mass dm);

$$dA = dpv = pdv + vdp = vdp,$$

therefore the power developed

$$d\pi = \frac{dA}{dt} = \frac{vdp}{dt}$$

and the brisance

$$B = \frac{d\pi}{v} = \frac{dp}{dt}, \frac{\sim \Delta p \text{ max}}{\Delta t \text{ max}}$$

which is the second definition.

It has to be borne in mind that the value of brisance depends upon the quantity of the decomposing substance insofar as $\Delta p_{max.}$ and $\Delta t_{max.}$ depend upon it under the experimental conditions (e.g., confinement).

As $B = \Delta p / \Delta t$ (second definition), it can be calculated from $\Delta p_{max.}$ and the corresponding $\Delta t_{max.}$, determined, e.g. from the detonation velocity D.

In Table I, this relation is applied to some high explosives according to the data of Schmidt[3,4] and of Paterson[5].

Brisance of High Explosives

Explosive	Loading Density	Brisance. 10^{-10} (in atm./sec.) (second definition)
P. E. T. N.	1.0	6.67
	1.5	26.2
Tetryl	1.0	6.36
	1.5	20.0
T. N. T.	1.0	4.42
	1.5	13.7

A good practical unit of brisance would, therefore, be:

$$10^{10} \text{ atm./sec.} = 10^9 . 1.01325 \text{ kw/1.}$$

Having thus established an absolute measure of brisance, we may, in principle, compare all gas evolving reactions, the decomposition of high explosives as well as that of propellants or even of non-explosive, non-combustible systems.

For propellants, the burning rates of British Cordites, (at 10 tons/sq. inch)[6] give the brisance values listed in Table II.

TABLE II

Brisance of Cordites (B in atm./sec.)

Designation	HW	HSC	W	SC	RDQ	Bofors	RDNA
$B.d^{-1/8}.10^{-7}$	6.22	5.96	4.63	3.92	3.30	3.06	2.51

($d^{-1}/3$ is near to, but somewhat smaller than, unity)

The brisance of high explosives is about 2000 times greater than that of Cordites.

To give another example, the *isothermic* decomposition of 100 mg of potassium perchlorate in vacuo at 600°C, leads to a "pseudo-brisance" of 0.53, and at 550°C of 0.008 units g.c.s.

Some improvement of these general calculations can still be envisaged, viz. by consideration of the influence of heat losses, demonstrated by Muraour;[7] they are less felt for high explosives (detonation velocity $\sim$ 5.10^5 cm/sec) than for propellants (decomposition rate $\sim$ 10 to 10^3 cm/ sec), as the heat losses increase with time. One may perhaps introduce an adiabaticity coefficient a ($s \leqslant a \leqslant 1$), and the notion of brisance under adiabatic conditions, B_s,

$$B = a \left(\frac{dp}{dt} \right)_s = aB_s \qquad B \gg B_s$$

the derivation being carried out under strictly adiabatic conditions, when $a = 1$, $B = B_s$. a is a function of time and goes to unity for $t \to 0$, and to 0 for infinitely slow, non-isolated systems.

A. E. SIMCHEN
Scientific Department,
Ministry of Defence

REFERENCES

1. BOLLÉ, 1927, *Jahresber. d. Techn. Reichsanst.*, **6**, 34.
2. CRANTZ, 1926, *Ballistik*, Springer, Berlin, **2**, 106.
3. SCHMIDT, 1935, *Ztschr. Schiess- u. Sprengstoffwesen*, **30**, 364.
4. SCHMIDT, 1936, *Ztschr. Schiess- u. Sprengstoffwesen*, **31**, 8.
5. PATERSON, 1949, *Research*, **1**, 221.
6. Ministry of Supply, 1951, *Internal Ballistics*, p. 37.
7. MURAOUR, 1922, *Mem. Artill. Franc.*, **3**, 339.

The Mechanism of the Fractionation of Aerosols in the Cascade Impactor

The Cascade Impactor permits the determination of the particle size and of the size distribution in an aerosol. The apparatus consists of a number (4—5) of cells, each of them containing an inlet, an orifice, a microscope slide, opposite the orifice, on which the particles collect, and an outlet perpendicular to the inlet. The orifices become increasingly narrower from the first to the last cell. The cells are connected with each other so that the outlet of one is joined to the inlet of the next one; the inlet of the first cell is directed towards the generator of the aerosol, the outlet of the last cell is connected to a pump which sucks the aerosol through the apparatus. The velocity of the jet increases from orifice to orifice; the largest particles collect on the first slide, smaller ones on the second, and so forth.

There exist two hypotheses in order to explain this fractionation process. The first one assumes that the effect is of purely aerodynamic nature, and that every particle which strikes a slide remains on it. To all particles the equation of May[1] applies:

1) $\varrho VD^2 / \eta l = \text{const.}$

(ϱ density of the particle, η its viscosity, V velocity of the jet, D radius of the particles retained, l length of the orifice); the constant is dependent on the impact yield n (number of particles retained in a cell: number of particles entering). As $Vl = \text{const.}$ (law of continuity) and as ϱ / η is a constant parameter for a given aerosol,

2) $n = f \ (D/l).$

Smaller particles give a smaller impact yield.

The second hypothesis assumes that also those particles strike the slide, which afterwards continue to flow into the next cell; they behave like

elastic balls (bounce-off effect). This hypothesis argues that the energy of a particle is composed of kinetic energy $\frac{1}{2}mV^2$ and surface energy $4\pi r^2\sigma$. When the particle strikes the slide, it is deformed and its kinetic energy is converted into surface energy, the increase of the latter being

$$3)\quad \triangle S = \tfrac{1}{2}mV^2$$

σ being the surface tension and $\triangle S$ — the increase of surface. If $\triangle S$ is greater than a threshold value, the particle will continue to flatten and remain on the slide; otherwise it will return to the spheric form, and the surface energy will be transformed into kinetic energy, but in the opposite direction. For the particles retained on the slide, one obtains by development of formula (3)

$$4)\quad DV^2 = \text{const.}$$

In order to decide between the two hypotheses, the path of the particles in the Impactor was photographed. According to the first hypothesis, these paths should become hyperbolic in the nighbourhood of the slides. According to the second, the path should be angular ($<$).

The apparatus used consisted of four parts: the Impactor, the aerosol generator, the illuminating unit and a camera.

The Impactor chamber was a brass cube, into which in two perpendicular directions holes of 30 mm radius were bored; another hole of the same diameter was bored into the fifth side of the cube. The holes in one direction were covered with flat, transparent glass slides through which the chamber could be illuminated and photographed; the glass slides in turn were partly covered with diaphragms so that only the part of the chamber between the orifice and the opposite slide was illuminated. One of the perpendicular holes was connected, through a flowmeter, with the pump, whilst the other two served for the introduction of the orifice (in the direction of the flow or perpendicular to it). To the orifice (rectangular cross-section) there was joined the slide on a little stand so that it was possible to vary the distance between the two.

The aerosol was generated with a Chicago Atomiser, giving particles of $1-100\mu$. In order to prevent disturbances through turbulence, the Atomizer was inserted into one neck of a large three-necked flask, the impactor directed to another opening.

The illuminating unit was a flash-light type instrument with a peak intensity of 2×10^7 lumen. The time of illumination was 1/1500 second; it was adjusted to the velocity of the particles, so that it was possible to observe on each photograph the beginning and the end of the path of the particles, and to measure its length in that period. The illuminating unit was arranged so

that only light dispersed by the aerosol particles could enter the camera.

The camera was an ordinary Leica with Elmar objective. Photographs were made with varying magnifications (1:1 to 1:4) and with different widths of the diaphragm so that the depth of focus changed. All photographs were taken in a dark room; the time of exposure was equal to the time of illumination.

The photographs (see the reproduction given below) showed clearly that the fractionating effect in the Impactor is of aerodynamic nature. The larger particles, of greater inertia, strike the slide opposite the orifice and are retained, whilst the smaller ones move in the direction of the flow and generally never reach the slide.
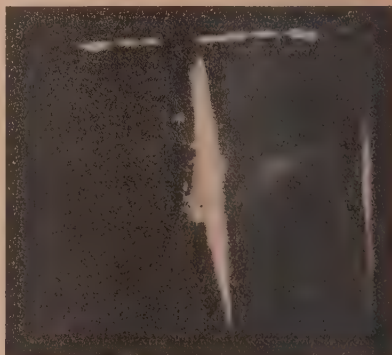
On the principle that the curvature of the paths of smaller particles is greater than that of large ones, a new and improved type of Cascade Impactor can be designed. One apparatus, which has been constructed, consists of a tube into which series of wedges of increasing opening angle were introduced. Wedges of small angles retain the larger particles, wedges of larger angles the smaller ones. A still simpler form of this apparatus would consist of a curved tube only, and represent a kind of mass spectrograph. According to the radius of curvature, particles of different size would be fractionally retained on different parts of the tube.

Y. SANDLER
A. DEMIEL (SCHWEIGER)
*Scientific Department,*
Ministry of Defence

REFERENCES

1. MAY, 1945, *J. Sci. Instr.*, **22**, 187.

The Reactions of Ceric Salts with Hydrogen Peroxide

In the catalytic decomposition of hydrogen peroxide by metal ions, e.g., in the case of Fenton's reagent by ferrous and ferric salts, OH and HO_2 radicals are formed, the reactions of which cannot be readily separated from each other.

In the present work, the reactions of ceric sulphate and ceric perchlorate salts with H_2O_2 were followed by determining the amounts of ceric salt reduced, hydrogen peroxide decomposed and oxygen evolved. It was shown, that in the pH range of 0—1.4 the reaction with ceric sulphate can always be described by

1. $H_2O_2 + Ce^{4+} \longrightarrow HO_2 + H^+ + Ce^{3+}$

followed by

2. $HO_2 + Ce^{4+} \longrightarrow H^+ + O_2 + Ce^{3+}$
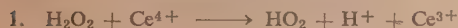
Even in the presence of large excesses of H_2O_2 the reaction

3. $H_2O_2 + HO_2 \longrightarrow H_2O + O_2 + OH$
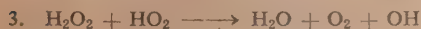
does not take place to any extent, and the radical OH does not appear in the course of the reaction. It was also shown that reoxidation of the Ce^{3+} salt formed did not occur at all. Thus the use of this reagent enables one to study the reactions of the HO_2 radical alone. Such reactions with organic substrates are now being investigated. In the case of ceric perchlorate solutions the reaction is complicated by the formation of a relatively stable complex of, probably Ce^{4+} and HO_2, e.g. $(CeHO_2)^{3+}$, which is now being investigated.

SHALOM BAER
GABRIEL STEIN
Department of Physical Chemistry.
Hebrew University, Jerusalem

The Behaviour of Ionized Polyelectrolyte Gels in Salt Solutions

An investigation of polyelectrolyte gels in inorganic salt solutions is of interest since it may throw some light on the behaviour of biological systems and on synthetic ion exchangers. The classical work on *non-polar* gels[1] has shown that their behaviour is completely defined both by their interaction constant with the swelling medium and by their degree of cross-linking. However, the behaviour of *polyelectrolyte* gels is in addition determined by their degree of ionization and ionic strength. The swelling of gels in pure water at different degrees of ionization has already been investigated[2]. The present work deals with the behaviour of ionized polyelectrolyte gels in equilibrium with aqueous solutions of univalent electrolytes. The distribution of ions between solution and gel phases and the potentiometric titration of the equilibrium solution were studied. For theoretical evaluation of the results, the degree of swelling of the gels at the different ionic strengths and degrees of ionization was determined as well.

The experiments were performed on polymethacrylic acid gels cross-linked by divinylbenzene. The degree of polymerization Z (between adjacent cross-links) was found to be 195, according to the procedure described by Katchalsky and Eisenberg[3]. These gels were brought to different degrees of neutralization by adding sodium hydroxide and were immersed in aqueous solutions of sodium chloride. After equilibrium had been established, the pH of the solutions was measured and both gel and solution were analysed for chloride. In addition the swollen surface-dry gels were weighed and the degree of swelling V determined.

The activity factor f_{NaCl}^{gel} was calculated from the distribution data according to the Donnan equation, the activity factor of the chloride in solution $f_{NaCl}^{solution}$ being known:

$$a_{NaCl}^{gel} = a_{NaCl}^{solution}$$
$$\frac{c_{NaCl}^{gel}}{c_{NaCl}^{solution}} = \frac{f_{NaCl}^{solution}}{f_{NaCl}^{gel}} \tag{1}$$

The activity factor in the gel was found to be much lower than that of the solution, having values as low as one half. Obviously the marked deviation from unity renders approximate calculations using concentrations instead of activities to be of little value even as a first approximation. The decrease of the activity factor is due to the strong attraction of the small gegen-ions for the ionized polyelectrolyte of the gel. The following expression for the activity factor was derived theoretically:

$$-\ln f = \frac{\nu^2 \varepsilon^2}{DkT} \frac{C_m}{h_o (V^{1/3} + \varkappa h_o/6) \Sigma C_i} \tag{2}$$

where ν denotes the number of fixed charges between two adjacent cross-links on the gel forming polymer, ε is the charge of an electron, D the dielectric constant of the gel, h the Boltzmann constant, T the absolute temperature, h_o is the mean distance between adjacent cross-links in unionized gel, V is the volume ratio of swollen to dry gel, C denotes the concentration of polymer in monomoles, C_m is the sum of free ion concentrations and $\varkappa$ denotes the inverse of the radius of the Debye ionic atmospheres.
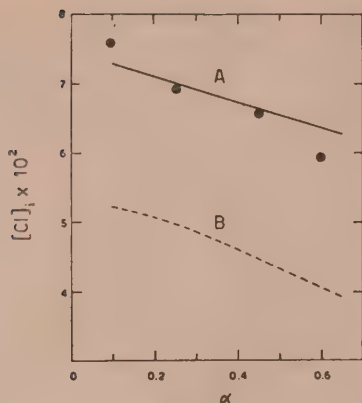
Figure 1

Salt concentrations in polymethacrylic acid gels of different degrees of ionization (α) in equilibrium with an external salt solution of 0.11 M NaCl.
A — full circles-experimental results; solid line-theoretical.
B — dotted line calculated from Donnan's equation with activity factors assumed equal to unity.

Figure 1 shows the concentration of chloride ion in the gel at different degrees of ionization, in equilibrium with 0.11 M NaCl solution. The points represent experimental results while the full curve A is derived from the theoretical values of the activity coefficient. For comparison the dotted curve B calculated according to the approximate Donnan equation using concentrations instead of activities, is given. It is readily seen that the experimental values are about 50 per cent higher than those calculated without taking into account the deviation of the activity factors from unity. On the other hand, the polyelectrolyte theory succeeds in describing the experimental behaviour.

The hydrogen ion concentration in the salt solution depends on the degree of ionization of the gel in equilibrium with that solution. The hydrogen ion distribution, in contrast to that of the sodium and chloride ions, is determined not only by the Donnan equilibrium but also by the dissociation constant of the gel forming polyacid. Potentiometric titrations of gels have been described in the literature for proteins and other natural polymers, as well as synthetic ion exchangers[4]. Information about the functional groups in the gel may be obtained from such titrations.

The generalized theoretical equation proposed here is based on three factors, (a) the Donnan distribution, (b) the electrostatic potential due to the fixed charges on the polyelectrolyte, and (c) the existence of a covalent bond between the hydrogen ion and the polymeric molecules of the gel, which can be expressed by an intrinsic dissociation constant K_0. The following equation

relates the pH measured in solution, with the degree of ionization α of the gel, the potential ψ, and the chloride ion activities in gel and solution.

$$pH = pK_0 - \log \frac{1-\alpha}{\alpha} + \log \frac{Cl_{sol}}{Cl_{gel}} + 0.4343 \frac{\varepsilon\psi}{kT} \quad (3)$$

If the polyelectrolytic nature of the gel is neglected by omitting the term containing ψ, erroneous results are obtained for pK_0. The value of the potential is found from theoretical considerations to be

$$\psi = \frac{2\nu\varepsilon}{Dh_0 V^{1/3}} \ln \left(1 + \frac{6}{h_0 \varkappa} V^{1/3}\right) \quad (4)$$
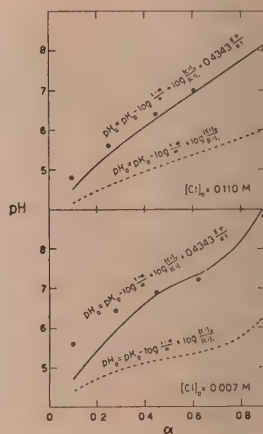
Figure 2

Potentiometric titration of swollen polymethacrylic acid gels (Z-195) in the presence of 0.007 M NaCl and 0.110 M NaCl in exterior solution. Full circles indicate experimental results; solid curves are calculated from equations (3) and (4) with inclusion of the potential correction; dotted curves—calculated ideal curves for the corresponding gels and degrees of swelling with neglect of the potential term.

The notation used is the same as in equation (2). Figure 2 shows the experimental points as well as the theoretical curves calculated according to equations (3) and (4), the values of pK_0 — 4.86 being in agreement with similar potentiometric titrations in solution.

The derivation of the equations describing the equilibrium of polyelectrolyte gels with aqueous solutions of electrolytes, as well as a full description of experimental results will be published elsewhere.

A. KATSCHALSKY
I. MICHAELI
Department of Polymer Research,
Weizmann Institute of Science,
Rehovot

REFERENCES

1. a) FLORY. P. J., 1944, *Chem. Rev.*, 35, 51.
   b) KUHN. W., PASTERNAK. R. and KUHN. H., 1947, *Helv. Chim. Acta*, 30, 1705.
2. KATCHALSKY A., LIFSON S. and EISENBERG H., 1951, *J. Polymer Sci.*, 7, 571.
3. KATSCHALSKY A. and EISENBERG H., 1951, J. *Polymer Sci.*, 6, 145.
4. a) LLOYD, D. and SHORE, A., 1938, "Chemistry of Proteins".
   b) SOOKNE, A. M. and HARRIS, M., 1940, *J. Research Natl. Bur. Standards*, 25, 47.
   c) TOPP, N. E. and PEPPER, W., 1949, *J. Chem. Soc. London*, 3300.
   d) HALE, D. K. and REICHENBERG, D., 1949, *Discussion Farad Soc.*, 7, 82.

Infrared Dispersion of Liquids

The induced electric moments of molecules and the effective charges on constituent atoms may be computed from the absolute intensities of absorption bands in the infrared spectrum. Absolute intensities as distinct from relative intensities are very hard to determine experimentally.

Optical dispersion in the infrared, which can be measured accurately, provides information which is, to a large extent, equivalent to that obtained from intensity' measurements. The dispersion of liquids in the infrared has been measured by the straightforward technique of prism refractometry using a hollow prism filled with the specimen. The optical path through such a prism is necessarily long and for this reason the method can be applied only in the case of fairly transparent liquids. Data have been published [1,2] for CCl_4, CS_2, $CHCl_3$ and $CHBr_3$. For organic and other strongly absorbing materials, an interferometric method has often been suggested [1,3], in which a thin film of the sample is held between two optical flats. Some work 'along these lines has been done. A few refractive indices in the region 1 to 15 μ have been measured with an accuracy of about one unit in the second decimal place [4].

We have now developed a refined method, based on our experience with the infrared interference spectrometer [5], using a Fabry-Perot interferometer with the sample between its plates. An accuracy of 0.001 has been achieved for dispersion. A programme of measurements on a series of organic liquids in the frequency region of the C-H stretching vibration has been initiated. Values of the effective charges for this vibration on the carbon and hydrogen atoms are being calculated.

A full account of the optical method will be published elsewhere.

J. H. JAFFE
U. OPPENHEIM
Department of Optics,
Weizmann Institute of Science,
Rehovot

REFERENCES

1. PFUND, 1935. *J. Opt. Soc. Am.*, 25, 351.
2. PITTMAN, 1939, *J. Opt. Soc. Am.*, 29, 358.
3. ROLLEFSON and ROLLEFSON, 1935, *Phys. Rev.*, 48, 779.
4. RAMADIER-DELBES, 1951, *C. R.*, 232, 1920; 1952, *J. Phys. Rad.*, 13, 47.
5. JAFFE, 1951, *Nature*, 168, 381.

Investigations in Statistical Mechanics of Ionized Polymeric Molecules

We propose a new approach to the determination of thermodynamic properties of polymeric molecules. The polymeric molecule is supposed to have N statistical segments, each of which carries ionic or molecular groups which interact with other groups. We assume that the statistical segments interact with each other, either through the attractive or the repulsive forces.

Ler r_i denote the position vector of the segment i. The distance between the two ends of the polymetric molecule will be

$$\vec{R} = \sum_{i=1}^{i=N} \vec{r_i} \tag{1}$$

Let us now suppose that each segment is displaced from its equilibrium position. The individual displacement is

$$\Delta \vec{r_i} = \vec{r_i} - <\vec{r_i}>_{av} \tag{2}$$

We ask what is the probability that N segments will suffer these displacements simultaneously. The element of the probability function will be

$$dW = Ae^{-U(\vec{r_1}\ldots \vec{r_N})/kT} d_\Delta \vec{r_i} \ldots d_\Delta \vec{r_N} \tag{3}$$

A is a constant 'and U is the potential energy of a system of N segments.

U/kT is also a change in entropy, $\Delta S/k$, arising from the fluctuations of the vectors $\vec{r_i}$ from their equilibrium positions. We assume that these fluctuations are small. Since ΔS has its minimum at the equilibrium, its derivative with respect to vectors $\Delta \vec{r_i}$ is zero at $\vec{r_i} = <\vec{r_i}>_{av}$. We develop ΔS in Taylor series. Then

$$U/kT = \Delta S/k = \frac{1}{2} \sum_{i,\ k\ 1}^{i,k=N} \lambda_{ik} \Delta \vec{r_i} \Delta \vec{r_k} \tag{4}$$

Transforming equation (3) as a biquadratic form one can show that

$$\lambda_{ik} = \frac{\partial^2 S}{\partial \vec{r_i}\ \partial \vec{r_k}}; \quad <\Delta \vec{r_i} \Delta \vec{r_k}>_{av} = \frac{1}{2} \frac{|\lambda|_{ik}}{|\lambda_{ik}|} \tag{5}$$

and

$$A = \sum_{i,\ k=1}^{i,\ k=N} \frac{|\lambda_{ik}|}{(2\pi)^{N/2}} \qquad (6)$$

$|\lambda_{ik}|$ is the determinant made from λ_{ik}; $|\lambda|_{ik}$ is the coactor of λ_{ik} in determinant $|\lambda_{ik}|$. Therefore

$$dW = \sum_{i,\ k=1}^{i,\ k=N} \frac{|\lambda_{ik}|}{(2\pi)^{N/2}} \exp \qquad (7)$$

$$\left\{ - \sum_{i,\ k=1}^{i,\ k=N} \lambda_{ik}\ \vec{\Delta r_i}\ \vec{\Delta r_k} \right\} d\vec{\Delta r} \ \ldots \ d\vec{\Delta r_k}$$

This is the general expression for small displacements of segments.

Suppose now that we are dealing with polymeric chains that are also Markoff chains. A special case of the Markoff chains is a chain of links whose individual displacements are independent of each other.

For such a chain the equation 7 greatly simplifies. We obtain, through the use of definitions (1) and (2)

$$W(R)dR = \frac{1}{\left[2NA^2 <\cos^2\Theta>_{av} \right]^{3/2}} \exp$$

$$\left[\frac{\left(R - NA^2 <\cos\Theta>_{av} \right)^2}{2NA^2 <\cos^2\Theta>_{av}} \right] R^2 dR \qquad (8)$$

A is the length of the statistical segment. If the segments do not interact with each other,

$$<\cos\Theta>_{av} = 0,\ <\cos^2\Theta>_{av} = 1/3$$

and the equation (8) reduces to the known expression derived for the rubber-like polymers.

$$<\cos\Theta>_{av} \text{ and } <\cos^2\Theta>_{av}$$

depend upon the inter-segmental potential energy. If we assume Boltzmann distribution of probabilities, then

$$<\cos\Theta>_{av} = \frac{\displaystyle\int_0^\pi \cos\Theta e^{-U(\Theta)/kT} \sin\Theta\, d\Theta}{\displaystyle\int_0^\pi e^{-U(\Theta)/kT} \sin\Theta\, d\Theta} \qquad (9)$$

$$\cos^2\Theta_{av} = \int_0^\pi \cos^2\Theta e^{-U(\Theta)/kT} \sin\Theta\, d\Theta$$

$$\bigg/ \int_0^\pi e^{-U(\Theta)/kT} \sin\Theta\, d\Theta$$

In the case of short-range forces, like hydrogen bonds, the formulae (9) are exact. In the case of long-range forces we have to substitute $\overline{U}$, the potential of average forces which act between two segments, for U. In the case of electrostatic forces the first approximation will be the Debye-Hueckel limiting form. The equation (8) will give us some of the properties of the polymeric molecules:

1) R_0, the most probable end to end distance

Since, by definition

$$\frac{d\ln (R_0{}^2 W(R_0))}{dR_0} = 0 \qquad (10)$$

we obtain

$$R_0 = \frac{1}{2} NA <\cos\Theta>_{av} +$$

$$\sqrt{N^2 A^2 <\cos\Theta>_{av}^2 + 4NA^2 <\cos^2\Theta>_{av}} \qquad (11)$$

2) In order to evaluate the free energy of the polymeric molecule, we assume a small ionization so that

$$<\cos\Theta>_{av} \ll 1,\ <\cos^2\Theta>_{av} \simeq \frac{1}{3}$$

Then

$$W(R)\ dR = CR^2 dR \exp$$

$$\left\{ -\frac{3}{2} \frac{R^2}{NA^2} + \frac{3}{2} \frac{R}{A} <\cos\Theta>_{av} \right\} \qquad (12)$$

The first term of the right side is the entropy term. The free energy of the molecule will be

$$F = \frac{3}{2} \frac{R^2 kT}{NA^2} - \frac{3RkT}{2A} <\cos\Theta>_{av} \qquad (13)$$

The last term of equation (13) is the electrical contribution to the free energy.

Y. MAZUR
Department of Polymer Research,
Weizmann Institute of Science,
Rehovot

Potentiometry and Electrophoresis of Polyelectrolytes

Potentiometric titrations and electrophoretic measurements have been extensively used for the characterization of biocolloids, however a theory embracing both methods has not yet been developed. Considerable potentiometric work has been done in the field of synthetic polyelectrolytes, but no electrophoretic research has been reported. We have undertaken the electrophoretic investigation of synthetic polyelectrolytes and as a result a correlation was found between potentiometry and electrophoresis. By the com-

bined measurements it is possible to derive the dissociation constant of the functional acidic or basic group and thereby to characterize the electrochemical behaviour of many important biocolloids unequivocally.

A general potentiometric equation for polymeric acids has already been derived from theory[1,2,3]:

$$pH = pK_0 - \log \frac{1-\alpha}{\alpha} + \frac{0.4343}{kT} \left(\frac{\partial F_e}{\partial \nu} \right) \quad (1)$$

The analogous equation for the potentiometric behaviour of positively charged polybases is:

$$pH = pK'_0 - \log \frac{\beta}{1-\beta} - \frac{0.4343}{kT} \left(\frac{\partial F_e}{\partial \zeta} \right) \quad (2)$$

where pK_0 is the negative logarithm of the intrinsic dissociation constant charcteristic of the ionizable acidic group and independent of the ionic strength. pK'_0 is correspondingly related to the dissociation constant of the cationic acid, conjugate to the basic group of the polymer. α and β are the respective degrees of ionization of the polyacid carrying ν negative charges and the polybase with ζ positively charged groups. F_e is the electrostatic free energy of the polyion.

In order to apply equations (1) and (2) to describe the potentiometric behaviour of polyelectrolytes and to determine the value of pK_0, $(\partial F_e/\partial \nu)$ must first be evaluated.

The theoretical derivation of $(\partial F_e/\partial \nu)$ *is possible if the electrostatic free energy* F_e as function of ν is known. In the case of randomly kinked polyions with an end to end distance h, carrying ν elementary charges ε, suspended in a medium of dielectric constant D and ionic strength characterized by Debye's $\varkappa$, $(\partial F_e/\partial \nu)$ was found to be:

$$\left(\frac{\partial F_e}{\partial \nu} \right)_{\varkappa, h} = \frac{2\nu\varepsilon^2}{Dh} \ln \left(1 + \frac{6h}{\varkappa h_0^2} \right) \quad (3)$$

h_0 is the end to end distance of a hypothetical uncharged molecule similar in every other respect to the charged polyion.

The Electrophoretic Potential. $(\partial F_e/\partial \nu)$ is the change in the electrical component of the free energy of a polyacid resulting from the addition of a single ionized group to the molecule. This is identical with the electrical work done in adding a charge ε to the polyion with the surface potential ψ_0, i.e.

$$\varepsilon\psi_0 = \left(\partial F_e/\partial \nu \right)_{\varkappa, h} \quad (4)$$

Equation (4) correlates the potentiometric value of $(\partial F_e/\partial \nu)$ with the surface electrical potential which can be *determined electrophoretically.*

ψ_0 of a colloid particle is derived from the electrophoretic mobility by the general relation[4]

$$300u = D\psi_0/C\pi\eta \quad (5)$$

where u is the mobility in cm^2 volt^{-1} sec^{-1}, D the dielectric constant, ψ_0 the surface potential in e.s.u. and η the viscosity of the solvent. C is a factor that has been numerically evaluated[5] for spheres and cylinders, respectively, of various sizes and in solutions of different $\varkappa$'s.

Introducing equation (4) into (1), we obtain for polyacids:

$$pH = pK_0 - \log \frac{1-\alpha}{\alpha} + \frac{0.4343}{kT} \varepsilon\psi_0 \quad (6)$$

and from equations (2) and (4) for polybases:

$$pH = pK'_0 - \log \frac{\beta}{1-\beta} - \frac{0.4343}{kT} \varepsilon\psi_0 \quad (7)$$

Equations (5), (6) and (7) correlate potentiometric *pH* measurements with electrophoretic mobility.

Comparison of molecular potentials derived by the different methods. As an example, the surface potentials of polymethacrylic acid (P.M.A.) at different degrees of ionization were determined potentiometrically (from equation (1)), electrophoretically (from equation (5)) and calculated theoretically (by equation (3)). The electrophoretic mobilities of polymethacrylic ions of different α's were measured by the moving boundary method, using a Tiselius type electrophoresis apparatus. Potentiometric titration was carried out with a P.M.A. solution of the same polymer concentration and ionic strength.

TABLE I

Surface potential from theory, potentiometry and electrophoresis.

0.1 base moles/lit. P.M.A. $\chi = 10^7$. $t = 15^{\circ}C$

	ψ_0 in e.s.u. x 10^4		
	theor.	potent.	electrophor.
0.097	1.12	1.10	0.89
0.15	1.36	1.31	1.13
0.25	1.68	1.65	1.51
0.36	1.93	1.88	1.75
0.63	2.46	2.40	2.50
0.72	2.62	2.55	2.62
0.82	2.79	2.74	2.74
0.97	3.05	2.93	2.75

As is seen from Table I the agreement between the theoretical, potentiometric and electrophoretic potentials is good.

Application of combined potentiometry and electrophoresis for the determination of the intrinsic dissociation constant of polyamino-acids: Polyaspartic-acid[6] was chosen as a model of an acidic polyamino-acid. Electrophoretic (m.b. method) and potentiometric measurements were carried out at an ionic strength of 0.5. pK_0 of

polyaspartic-acid has been evaluated from these combined measurements using equations (5) and (6). It was found to be quite constant for different α's and its mean value is $pK_0 = 3.53 \pm 0.06$.

Polylysine[7] is a typical polymeric base: its apparent pK_a increases with increase of ionic strength. Here again, a direct evaluation of pK_0 was achieved by combined electrophoretic and potentiometric measurements carried out at the same ionic strength ($\mu = 0.5$) and by the use of equation (7) and (5). The mean value was found to be $pK'_0 = 10.44 \pm 0.07$. This value corresponds to a basic dissociation constant of $pK_b = 3.56$ and is practically equivalent to that of butyl amine with $pK_b = 3.53$.

The constancy of the intrinsic dissociation constant, pK_0, for the whole range of degrees of ionization and its close correspondance to the repeating monomeric unit of the polyelectrolyte have been proven. This gives strong support to the applicability of the combined method for the electrochemical characterization of ionizable biocolloids.

A more general treatment of the dissociation behaviour of a series of polyacids and polybases, both natural and synthetic, is given elsewhere.

AHARON KATCHALSKY
NEHEMIA STERNBERG
(SHAVIT) *
Dept. of Polymer Research,
Weizmann Institute of Science,
Rehovot

* Part of a thesis submitted by N. Sternberg (Shavit) to the Senate of the Hebrew University, Jerusalem, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

REFERENCES

1. OVERBEEK, J. TH. G., 1948, *Bull. soc. chim. Belges,* 57, 252.
2. KATCHALSKY, A. and GILLIS, J., 1949, *Rec. trav. chim.,* 68, 879.
3. KATCHALSKY, A. 1951, *J. Polymer Sci.,* 7, 393.
4. ABRAMSON, H. A., MOYER, L. S. and GORIN, M. H., 1942, *Electrophoresis of Proteins.* Reinhold Publishing Corp., New York, Chap. V.
5. OVERBEEK, J. TH. G., 1950, *Advances in Colloid Science,* Interscience Publishers Inc., New York, Vol. III.
6. BERGER, A. and KATCHALSKY, E., 1951, *J. Am. Chem. Soc.,* 73, 4084.
7. KATCHALSKI, E., GROSSFELD, I. and FRANKEL, M., 1948, *J. Am. Chem. Soc.,* 70, 2094.

The Surface Properties of Aqueous Solutions of Polyelectrolytes

Considerable work has been done on the surface adsorption of natural polyelectrolytes and especially proteins[1]. Yet no satisfactory interpretation of the adsorption mechanism has been given owing to the complex structure of these substances and their tendency to coagulate at the surface.

The simpler case of adsorption of synthetic polyelectrolytes in aqueous solutions has been recently investigated. For the case of unionized polymers a full theoretical description of the behaviour has been given.

The aim of the present paper is to broaden the scope of the investigation to include amphoteric ionized copolymers which may serve as simple models of the surface behaviour of proteins. The surface properties of six copolymers of methacrylic acid (M.A.) and 2-vinyl-pyridine (V.P.) were studied by us. The molar percentage proportion of V.P. in each was respectively 21%, 30.6%, 41%, 52%, 63%, 73%.

The surface tension and surface potentials were measured as functions of the concentration of copolymer, of added salts and of the pH of the solution. The surface tension was measured by the stalagmometric method developed by us, at a temperature of $28 \pm 0.1^\circ$.

The surface potential was measured with a polonium electrode against a calomel electrode using a Beckman model G pH meter.

V.P. is more surface active than M.A.; hence one would expect the copolymers richer in the former to lower the surface tension of water more than those richer in M.A. Figure 1 indicates that this is verified experimentally. The surface tension lowering is greatest near the isoelectric point (I.P.) at pH 4—6 and is less on either side of I.P. The diminution of the surface activity of the polyampholyte on both sides of the I.P. is due to the change of the surface potential (Figure 2) and the lowering of the van der Waals adsorption energy, with ionization.

The curves of Figure 1 are not symmetrical about the isoelectric point. Investigating the influence of the ionic strength it was found that on lowering the ionic strength the symmetry of the curves
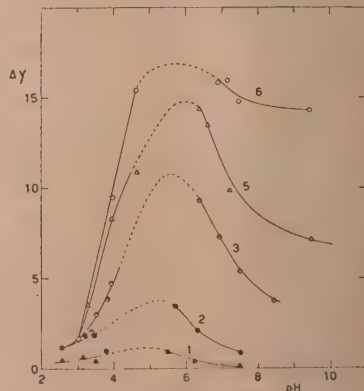

Figure 1

Dependence of surface tension lowering (γ) on pH.
The copolymers: 1—21% V.P.; 2—30.5% V.P.; 3—41% V.P
5—63% V.P.; 6—73% V.P.
Polymer concentration: $C_p = 0.005$ mono-Mol/l
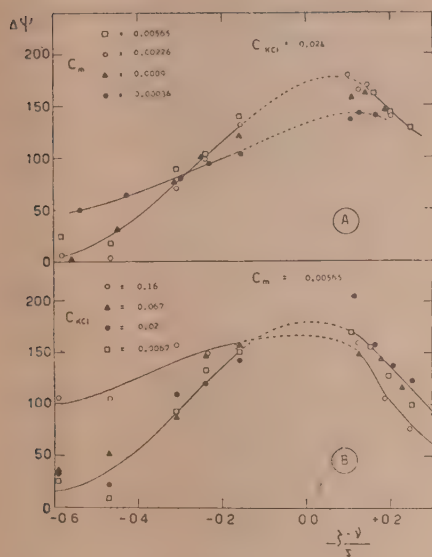concentration of KCl: $C_l = 0.02$ M/l

Figure 2

Dependence of surface potential on the net charge of the polyampholyte (No. 3)
A — Various polymer concentrations
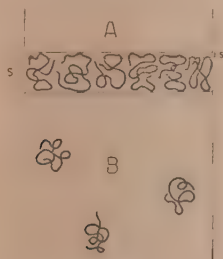B — Various KCl concentrations.

Figure 3
The phases

A — air; B — bulk phase; S — surface phase; s — adsorption layer.

increases while the slopes become more steep on both sides of I.P. This behaviour in the presence of salts is due to the fact that 2-vinyl-pyridine is a larger ion than the methacrylate ion and therefore the effect of added salts is more pronounced on the alkaline side than on the acid side. For the same reason the effect of divalent cations is greater than that of divalent anions.

The addition of salts at a constant concentration of polymer always increases the lowering of the surface tension. This lowering is proportional to the logarithm of the salt concentration. There is also a linear relationship between the surface tension and the logarithm of the ampholyte concentration in the presence of excess salt. In both cases the slope of the straight lines is related to the net charge of the adsorbed polyampholyte.

In order to express quantitatively the observed phenomena, the mechanism of adsorption can be pictured as follows: The solution can be divided into a bulk phase and a surface phase with a thickness of one polymer molecule. The surface phase itself can be subdivided into an adsorption layer a few Angstroms thick and the surface phase properly. Under the experimental conditions employed the adsorption layer is fully saturated with polymer segments.

We may imagine the process of the formation of the surface bulk equilibrium system to be composed of two steps: in the first step we increase the concentration of polymer and added salt in the bulk phase; simultaneously polymer molecules enter the surface phase in accordance with the thermodynamic potentials in both phases and with the van der Waals and electrical forces operating in the adsorption layer.

In the second step, after the adsorption layer is saturated, further increase in the bulk concentration does not influence the adsorption layer. Yet the concentration in the surface phase increases to maintain equality of thermodynamic potential with the bulk phase.

Due to Donnan equilibrium, the concentration of ions of charge similar to that of the polyampholyte, can be neglected in comparison to the concentration of the counter ions.

From these considerations the concentration of polymer in the surface is given by the equation

$$C_p{}' = \frac{f_i}{f_i{}'} \left(C_p{}^{o'} + C_i \right)$$

where C_i is the bulk concentration of added salt, $C_p{}'$ is the surface concentration of polymer, $f_i/f_i{}' =$ the ratio of the activity coefficients of the small ions in the bulk and surface. $f_i/f_i{}'$ is assumed to be constant under the conditions of the experiment. As in the bulk phase the salt concentration is large as compared with the polymer charge concentration $f_i{-}1$ whereas in the surface phase the ratio is reversed and $f_i{}' = 0.2$ [4].

$C_p{}^{o'}$ is the concentration of polymer in the surface phase at the end of the first step, assumed to be constant under the conditions of experiment.

From the equation, it is seen that the concentration of polymer in the surface rises linearly with the concentration of salt in the bulk phase.

Since the electrochemical potentials in the bulk and surface phases are equal and since the surface potential is nearly independent of the concentrations of the polymer and added salt

as shown by experiment (Figure 2) the following general relationship is obtained

$$- \triangle \gamma = RT \left(A \ln \frac{C_{i_2}}{C_{i_1}} + B \ln \frac{C_{p_2}}{C_{p_1}} \right)$$

where A and B are constant dependent only on the polymer constitution and its net charge. $\triangle \gamma$ is measured depression of the surface tension.

ISRAEL MILLER
AHARON KATCHALSKY
*Department of Polymer Research,
Weizmann Institute of Science,
Rehovot.*

REFERENCES

1. KATCHALSKY, A. and MILLER, I., 1951, *J. Phys. and Coll. Chem.*, 55, 1182.
2. CUMPER, C. W. N. and ALEXANDER, A. E., 1950, *Trans. Farad. Soc.*, 46, 243.
3. NEURATH, H. and BULL, H. B., 1938, *Chem. Rev.*, 23, 391.
4. KATCHALSKY, A., KEDEM, O. and EISENBERG, H., *J. Pol. Sci.*, (in press).

Gas Phase Reactions between Nitrogen Dioxide and Hydrocarbons

Three distinct modes of reaction have been observed between nitrogen dioxide and the higher hydrocarbons. When mixtures of hydrocarbons and nitrogen dioxide are admitted to a hot vessel, they may:
1. be oxidized slowly, or
2. give a blue luminescence during reaction, or
3. explode violently.

The slow reaction has been followed by a photoelectric light absorption method. The products of the reaction are: secondary nitroparaffins, organic nitrites, normal nitroparaffins, water, nitric oxide and some carbon monoxide and carbon dioxide. These products are consistent with the reactions of nitrogen dioxide as a free radical.

The presence of organic nitrites and their subsequent decomposition is important in the cool flame region, and the light emitted may be associated with the formation and reactions of the alkoxy radical.

The critical pressure required for ignition lies above that for cool flames. There is a short time lag of the order of several seconds prior to ignition. This same lag can be eliminated by the addition of several per cent of ethyl nitrate. These ignitions may be described as "degenerate explosions" following Semenoff's theory of chain reactions. The gases formed are (in decreasing abundance) nitric oxide, carbon monoxide, nitrogen, carbon dioxide, hydrogen, and nitrous oxide.

A. D. YOFFE
*Department of Isotope Research,
Weizmann Institute of Science,
Rehovot.*

An Analogy between certain Pentavalent Phosphorus Compounds and Corresponding Carbon Derivatives

In contrast to the situation obtaining in carbon chemistry, the phosphorus atom is able to use more than four orbitals to form bonds with other atoms. In a considerable number of pentavalent phosphorous compounds it has been shown that the central atom uses an additional orbital in addition to the usual hybridized s,p bonding orbitals.

Since in carbon compounds it is energetically impossible to increase even temporarily the number of bonding orbitals above four, all the substitution reactions use mechanisms which do not involve an expansion of the valency shell. Since in phosphorus such a limitation is not present, the possibility exists, *a priori*, that some additional reaction mechanisms may be available to this atom in its replacement reactions. The results of extensive kinetic investigations of certain pentavalent phosphorus compounds have failed to indicate the presence of any novel mechanism of substitution. This indicates the apparent inability of the phosphorus atom to use orbitals of the type sp^3d^2 in the transition state of its reactions and hence the limitation to mechanisms similar to those found in carbon chemistry. Experimentally, this fact is apparent by the great similarity which is found between substitution reactions of these phosphorus compounds and the analogous carbon derivatives.

J. DOSTROVSKY
M. HALMANN
*Department of Isotope Research,
Weizmann Institute of Science,
Rehovot.*

Free Energy and Colligative Properties of Solutions of Rod-like Polyelectrolytes.

The characteristic properties of polyectrolyte solutions depend largely on the contribution of the strong electrostatic field to the free energy of these solutions. The electrostatic repulsion between the parts of the polyelectrolyte molecules stretches the molecule in certain cases to a rod-like shape. Fuoss, Katchalsky and Lifson[1] have calculated the electrostratic potential ψ of the rod-like molecule as a function of the distance from the molecule, its degree of ionisation α, and the concentration C_m of the solute. The knowledge of the potential ψ makes it possible to calculate the free energy by a charging process which is a generalisation of Debye's charging process. One starts from a hypothetical reference state in which all the ions are supposed to carry no charge. The ions are then charged gradually, reversibly and isothermally.

The energy of charging is $\int_o^\varepsilon \psi d\varepsilon$ per ion, where

ψ is a function of ε and depends also on the distance from the rod-like molecule. The free energy of the whole solution is obtained by summing up the above charging energy over the ions in the solution, taking into account that their density varies gradually at every point during the charging process. It is found that the electrical free energy F_e is correlated to the inner energy U through the expression

$$F_e = \int_0^\lambda U \frac{d\lambda}{\lambda}$$

where λ is proportional to the degree of ionisation and is given by

$$\lambda = \frac{\nu \varepsilon^2}{DhkT},$$

ν being the number of ionised groups on the macromolecule and h its length. D, k, T, ε are the dielectric constant, Boltzmann's constant, temperature and elementary charge, respectively. The internal energy of the solution is evaluated explicitly, thus

$$U = Dh \left(\frac{kT}{\varepsilon}\right)^2 \left[(1+\beta^2)s + \lambda + \ln \frac{(1-\lambda)^2 - \beta^2}{1-\beta^2}\right]$$

s is a parameter representing the concentration according to:

$$s = \frac{1}{2} \ln \frac{1000}{V_m} - \frac{1}{2} \ln C_m$$

where $\overline{V}_m$ is the partial monomolar volume of the polymer, and C_m its concentration in monomoles per liter; β is an integration constant dependent on s and λ, according to

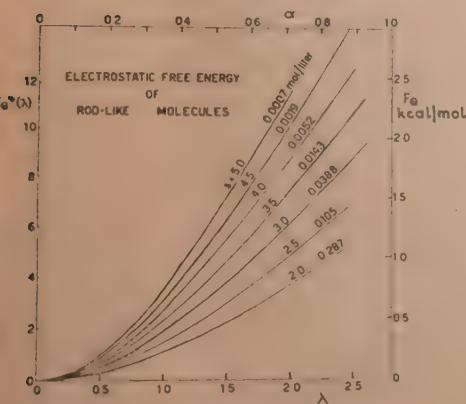$$\lambda = \frac{1-\beta^2}{1 + \beta \cot h \beta s}$$

Figure 1

The integration leading from the internal to the free energy was performed graphically (Figure 1). In the graph $F_e{}^*$ is defined by

$$F_e = Dh \left(\frac{kT}{\varepsilon}\right)^2 F_e{}^*$$

The right hand and upper scales describe the value of the electrical free energy in kcal per mole for polymethacrylic acid, α being the degree of ionisation. An example of the use of the theory is the calculation of the osmotic pressure of polymethacrylic acid which has been compared with Kern's measurements[2]. The osmotic coefficient appears to be

$$\varphi = 1 - \frac{1}{2\lambda} \frac{\partial F_e{}^*}{\partial s}$$
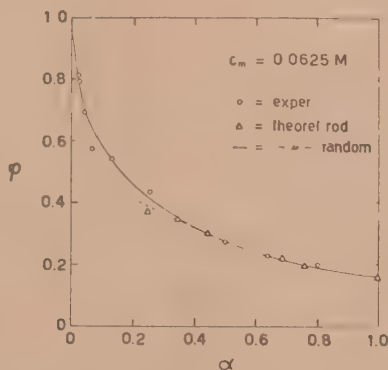
Figure 2

Figure 2 gives a picture of the agreement between theory and experiment. The extremely low value of the osmotic factor shows clearly the importance of the electrostatic effect, as compared with the case of simple electrolytes.

S. LIFSON
A. KATCHALSKY
Department of Polymer Research,
Weizmann Institute of Science
Rehovot.

REFERENCES

1. FUOSS, KATCHALSKY and LIFSON, 1951, Proc. Nationa
   Acad. Sci., U.S.A., 37, 579.
2. KERN, Ztschr. physikal. Chem., 1938, A181, 249.

Photochromy, a New General Method for the Study of Reversible Colour Changes of Organic Compounds.

Thermochromy is well known as the process of reversible colour changes on heating where no chemical process is involved. It is specific for a great number of derivatives of the bianthrone series[1,2,3], and for a number of spiran derivatives[4,5,6].

A great deal of effort has been devoted for the last twenty-five years, to the exploration of the

chemical and physical behaviour of these compounds, in order to throw some light on the origin and nature of this phenomenon. Till now no satisfactory explanation of the cause of thermochromism has been reported.

It was first found by the author in 1950[7] that various compounds of the bianthrone series are reversibly converted into coloured forms by irradiation at low temperatures. This new way of reversible colour change has been named 'photochromy'.

An extensive systematic study of all the known thermochromic compounds by this new method, with a view to investigating the relation of photochromy to thermochromy gave the following results[8,9]:

The coloured forms obtained either by heat or by irradiation at low temperature are identical.

In the latter process practically a complete conversion into the coloured form takes place.

It is evident that photochromy makes possible the investigation of the physical properties of the coloured forms at concentrations far exceeding those attainable thermally.

From a kinetic study of the reversion process 'coloured → colourless' it was found that this process involves a passage of a potential barrier of at least 12—20 kcal/mol. This indicates that the transformation from one form into the other involves changes in *atomic* configuration.

It is reasonable to assume that in the sterically hindered bianthrones which are only photochromic and not thermochromic, the energy difference between the non-coloured and the coloured forms is so large that within the accessible range of temperature no observable shift of the equilibrium towards the coloured forms can occur[10].

Coplanarity of the two halves of the molecule in the bianthrone series has no influence on the process of reversible colour change, since all the sterically hindered derivatives of bianthrone are photochromic[10].

In the case of xanthylidene-anthrone the increase in the dipole moment with intensification of colouration during irradiation at low temperature shows that a bipolar mesomer contributes materially to the structure of the coloured modification.

The spectral hypsochromic shift in the coloured forms in relation to non-coloured ones and the simultaneous decrease in the intensity of luminescence do not support the suggestion of the existence of free radicals as intermediates in the colouration process in the bianthrone series. The absence of phosphorescence in both the coloured and non-coloured forms furnishes further negative evidence in this regard.

Also in the spiran series there are derivatives which are thermochromic, e.g. 1:3:3-trimethyl-indoline-2-spiro-6'-(2':3'- *β*-naphthopyran), and others that do not change their colour on heating, like dibenzospiropyran. Both of them are reversibly converted into coloured forms by irradiation at low temperatures[11].

No increase in dipole moment in the thermochromic spiran was observed during colouration. This proves that (contrary to the current concept) there is no contribution of a bipolar mesomer in the coloured form of this spiran.

During colouration of this spiran by low temperature irradiation, its luminescence not only increases, but also shifts to longer wavelengths, as compared with the luminescence of the non-coloured form. Also some phosphorescence could be observed in the coloured form at —180°C in a rigid glassy medium. These facts suggest that during colouration of this spiran an intermediate state of a free radical is formed.

YEHUDA HIRSHBERG
Department of Optics,
Weizmann Institute of Science,
Rehovot.

REFERENCES

1. SCHLENK, W., *Ausfuehrliches Lehrbuch der Organischen Chemie,* Wien, 1939.
2. BERGMANN, E., 1948, *Isomerism and Isomerization of Organic Compounds,* New York
3. SCHOENBERG et al., 1946, *J. Chem. Soc.,* 442.
4. DICKINSON and HEILBORN, 1927, *J. Chem. Soc.,* 14, 1999.
5. KOELSCH, 1951, *J. Org. Chem.,* 16, 3038.
6. KNOTT, 1951, *J. Chem. Soc,.* 3038.
7. HIRSHBERG, 1950, *Compt. rend.,* 231, 903.
8. HIRSHBERG and FISCHER, 1952, *J. Chem. Soc.,* in press.
9. FISCHER and HIRSHBERG, 1952, *J. Chem. Soc.,* in press.
10. HIRSHBERG, LOEWENTHAL and BERGMANN, *Bull. Research Council of Israel,* 1, No. 1, 139.
11. BERGMANN, WEIZMANN and FISCHER, 1950, *J. Am. Chem. Soc.,* 72, 5009.

A New Type of Hydrogen Bonding in Ortho-substituted Benzaldehydes

One of the most conspicuous manifestations of the hydrogen bond is to be found in the infra-red spectrum; its close examination makes it possible to detect the hydrogen bond, to investigate its character and to study its strength[1,2,3].

These methods can also be used for the identification of the internal hydrogen bond which brings about chelation and occurs, e.g., in *o*-chlorophenol[4], salicylic aldehyde[5], ethanolamine[6], etc. These bonds, as hydrogen bonds in general, express themselves in a *decrease* in the normal frequencies of the groups partaking in the chelate ring, because of a certain weakening of their bonds as the result of the hydrogen bonding. In all those cases, which have been investigated hitherto, all the hydrogen atoms forming the hydrogen bond had been supplied either by a hydroxylic group, an amino group, HCl, HCN or the like, but never by C—H groups unless the carbon atom was heavily burdened by chlorine atoms[7].

During an investigation of the aldehydic absorption band, due to the $(O \rightarrow) C — H$ stretching mode of vibration which occurs in acetaldehyde at 2710 cm^{-1} [8] and in tetrahydrofurfural at 2710 cm^{-1} [9], it was found that, although benzaldehyde

absorbs normally at 2710 cm⁻¹, in *o*-nitrobenzaldehyde, this band rises surprisingly up to 2770 cm⁻¹. *o*-Chlorobenzaldehyde also has this band at 2770 cm⁻¹, 2,6-dichlorobenzaldehyde at 2780 cm⁻¹. *o*-methoxybenzaldehyde at 2755 cm⁻¹, and 2-ethoxy-1-naphthaldehyde at 2770 cm⁻¹. On the other hand, when the substituent is not in the ortho-position, the frequency is more or less the same as in benzaldehyde, independently of the electrical nature of the substituent:

p-anisaldehyde	2730 cm⁻¹
p-carboxamidobenzaldehyde	2735 cm⁻¹
p-dimethylaminobenzaldehyde	2730 cm⁻¹
p-nitrobenzaldehyde; terephthalic aldehyde	2740 cm⁻¹
p-cyanobenzaldehyde; *m*-nitrobenzaldehyde	2720 cm⁻¹
p-hydroxybenzaldehyde	2710 cm⁻¹

Also cinnamic aldehyde falls in line (2725 cm⁻¹).

This increase in the frequency of ortho-substituted aldehydes cannot be explained by a steric effect, as a molecular model shows *o*-chlorobenzaldehyde, which has the same frequency as the *o*-nitro-compound, to be free of *steric* interaction between the aldehydic hydrogen and the chlorine atoms. Nor can the frequency increase be attributed to the electronic effect of the substituents as the change of so different a group as the formyl one for the dimethylamino group does not bring about a change of more than 10 cm⁻¹ in frequency. The increase must therefore be explained as being due to an internal hydrogen bond between the chlorine atom or the oxygen atom of the nitro- or alkoxy group, in the ortho position, and the formyl hydrogen atom whose bond to the carbon atom is not too strong as evidenced both by its chemical reactivity (oxidation of an aldehyde into an acid; benzoin reaction) and by the comparison of its normal stretching frequency (2710 cm⁻¹) with that of a CH group (2890 cm⁻¹)[10]. This hydrogen bond is at about a right angle to the C—H bond as shown by the models of *o*-chloro- and *o*-alkoxy-benzaldehydes and by the X-ray diffraction pattern in the case of *o*-nitrobenzaldehyde. A stretching of the aldehydic C—H bond is, therefore, accompanied by a kind of bending of the hydrogen bond, and consequently, the frequency of this C—H vibration rises.

In order to test this explanation, *o*-toluic aldehyde was investigated. As no hydrogen bond can be expected to occur between a methyl and a formyl group, the C—H vibration must absorb normally in this case in spite of the fact that from a steric and electronic point of view this aldehyde is similar to some of the anomalous aldehydes. It was found, indeed, that its (O) C—H frequency lies at 2700⁻¹. This hydrogen bond may be used to explain the isomerisation of *o*-nitrobenzaldehyde

into *o*-nitrosobenzoic acid under the influence of light[11]

S. PINCHAS
*Department of Optics,
Weizmann Institute of Science,
Rehovot*

1. BADGER, R. M. and BAUER, S., 1937, *J. Chem. Phys.*, 5, 839.
2. KUHN, L. P., 1952, *J. Amer. Chem. Soc.*, 74, 2492.
3. HUNSBERGER, 1950, *J. Amer. Chem. Soc.*, 72, 5626.
4. VERGNOUX, A. M. and DADILLON, R., 1941, *Compt. Rend.*, 213, 166.
5. ERRERA, J. and SACK, H., 1938, *Trans. Farad. Soc.*, 34, 728.
6. BERGMANN, E. D., GIL-AV, E. and PINCHAS, S., 1952, *J. Amer. Chem. Soc.*, 74, (in press).
7. JONES and BADGER, R. M., 1951, *J. Amer. Chem. Soc.*, 73, 3132.
8. MORRIS J. C., 1943, *J. Chem. Phys.*, 11, 230.
9. 1951, *J. Institute of Petroleum*, 37, 119
10. FOX and MARTIN, *Proc. Roy. Soc.*, A 175, 208.
11. LEIGHTON, P. A. and LUCY, F. A., 1943, *J. Chem. Phys.* 2, 756.

The Influence of Pressure on the Alumina Catalysed Transformation of Alcohol to Ether

The classical method for the preparation of ether consists of the absorption of ethyl alcohol in sulphuric acid and subsequent hydrolysis of the ester formed. A second method in which alcohol vapours are passed over a catalyst, generally alumina, has not found technical application in spite of its obvious advantages, as it is accompanied by the formation of ethylene, an undesirable by-product. In the present study, the influence of pressure and other factors on the catalytic method has been investigated.

It is obvious from theory that increase of pressure will shift the equilibrium towards ether formation, as in the conversion of alcohol to ethylene two molecules, in that to ether only one molecule is formed. Under the usual operating conditions, however, we are very far from the thermodynamical equilibrium state. Nevertheless, the experiment showed that with increasing pressure, ethylene formation is significantly suppressed in favour of the synthesis of ether. Figure 1 summarises the results at 20 atm. and 355°. It has further been found that at 40 atm. and 335°, less than 1% of the alcohol is dehydrated to ethylene (Figure 2).
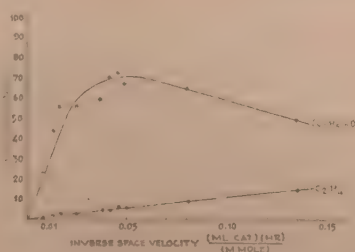

Figure 1

It is, therefore, clear that one can solve the technical problems by an increase of the operating pressure; however, a new and unforeseen difficulty made itself felt: whilst the addition of ether or ethylene to the charge of alcohol has no influence on the transformation of the latter, *water* has a very adverse effect. Figure 2 demonstrates the
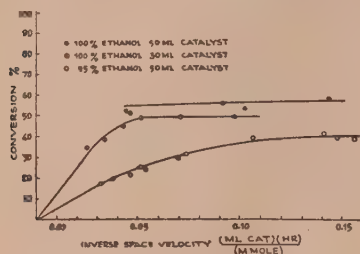
Figure 2

difference between the transformation of anhydrous and of 95% alcohol, and the addition of 20% water suppresses ether formation completely. The cause of this phenomenon is the hydrating effect of water on the catalyst, which is transformed from γ-alumina to α-alumina monohydrate. Figure 3 proves this theory. Catalyst samples were taken under
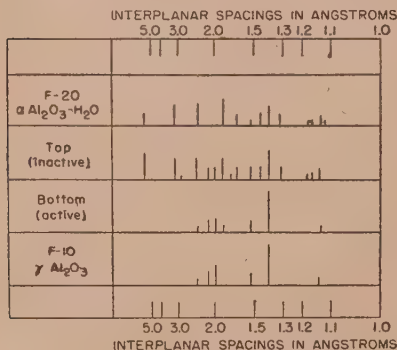
Figure 3. — X-ray diffraction pattern of alumina catalyst showing deactivation by conversion to α $Al_2O_3 \cdot H_2O$.

conditions of maximum conversion from the inlet (bottom) and from the outlet (top) of the reaction chamber, and investigated by the method of X-ray diffraction. In Figure 3, the intensity of diffraction is indicated by the height of the lines. One sees that at the inlet the alumina has the γ-form; at the outlet most of it is present in the form of the α-monohydrate. This effect will be most pronounced at high pressures, for which for a given conversion the partial pressure of water is high, so that hydration of the alumina is favoured.

These experiments lead to the conclusion that in the catalytic process too high pressures have

to be avoided because they lead to hydration of the catalyst, and too low ones, because they favour the formation of ethylene as by-product.

This investigation was carried out at Northwestern University, Evanston, Illinois under the auspices of the Point Four Programme.

H. FEILCHENFELD
Research Council of Israel,
Jerusalem

Oxygen Exchange Between Nitric Acid and Water as a Criterion for the Mechanism of Aromatic Nitration

The rate of exchange of oxygen atoms between nitric acid and water has been measured as a function of medium composition. The rate is shown to be given by:

$$R = \frac{3[HNO_3][H_2O]}{3[HNO_3]+[H_2O]} \cdot \frac{d}{dt} \ln(\alpha_\infty - \alpha) \quad (1)$$

in which α is the isotopic abundance of O^{18} in the nitric acid, defined:

$$\frac{[O^{18} \text{ in } HNO_3]}{3[HNO_3]}$$

Expression (1) yields the rate of the reaction:

$$HNO_3 + H_2O \longrightarrow HNO_3 + H_2O,$$

regardless of the isotopic labelling of the molecules, having taken into account all the forward and back reactions between the variously labelled species.

The rate of exchange, R, shows a high order dependence on nitric acid concentration.

The nitration of a number of aromatic compounds has been followed kinetically under identical conditions. It is shown that the rate of nitration is always less than R, but for very reactive compounds approaches it as a limiting value, the kinetic form changing at the same time from first to zero order.

The results are interpreted as indicating that oxygen exchange, literally the nitration of water, proceeds as follows:

$$2\ HNO_3 \underset{}{\overset{fast}{\rightleftarrows}} H_2NO_3{}^+ + NO_3{}^-$$

$$H_2NO_3{}^+ \underset{R}{\overset{R}{\longrightarrow}} NO_2{}^+ + H_2O$$
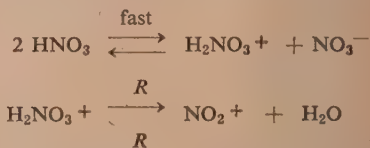
Similarly, nitration in aqueous nitric acid takes place by means of the nitronium ion, $NO_2{}^+$, and not, as has been suggested, by means of the nitracidium ion, $H_2NO_3{}^+$.

C. A. BUNTON
E. A. HALEVI
William Ramsay and Ralph
Forster Laboratories
University College, London

The Exchange Reaction Between Deuterium and Ammonia on the Surface of Metal Powders and Metal Wires

In order to clarify certain contradictions in the literature regarding the laws governing the metal-catalysed exchange of deuterium and ammonia[1,2,3,4], the exchange reaction on various metals, both in the form of powders and of wire, has been followed systematically using a static system for the investigation. The pressure range studied was 20—250 mm Hg, for both gases.

For the evaluation of the experimental results, an idealised equation:

light ammonia + heavy hydrogen $\rightleftarrows$
heavy ammonia + light hydrogen

was adopted, in which the term "light (heavy) ammonia" denotes *all* ammonia molecules which can exchange an H(D) atom for a D(H) atom. The molecule NH_2D, e.g., is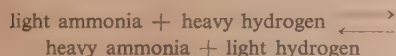 counted twice as light and once as heavy ammonia. It is further assumed that the reaction in both directions depends in the same manner on ammonia and hydrogen pressures, but that the rate is different by a factor ε due to the isotope effect (ε has been calculated to be 1.675 at 245°) — and thirdly that there is no difference in isotopic composition in the catalyst and in the gas phase, an assumption which has been shown experimentally to be reasonably correct.

On an iron powder, the exchange reaction proved to be independent (within 10%) of the ammonia pressure, and of first order in respect to deuterium; the apparent energy of activation was 17.5 kcal/mole. No inhibitory effect of the ammonia[3] could be detected under our experimental conditions.

On a second sample of iron powder, the activity decreased from run to run, but could be restored by heating the sample with pure deuterium at 350°, which may be ascribed to the formation of a surface amide, as suggested before. The activation energy with this sample was 19.8 kcal/mole.

On a nickel powder, some similar effects appear to be prevalent; in some cases, the exchange rate augmented as the reaction proceeded and seemed to increase with ammonia pressure.

The experiments carried out with other metal powders are summarised in Table I and those with metal wires in Table II. The low activation energy for palladium (Table II) is noteworthy and invites to further experiments with this metal. The activation energies found for nickel powder and nickel wire are in satisfactory agreement with each other.

It appears that in most cases, in which conclusions can be drawn from the experiments, the reaction is of first order with regard to hydrogen and of the order zero with regard to ammonia.

J. GUTTMANN
B. SCHNEIDMESSER
Scientific Department,
Ministry of Defense.

TABLE I
Deuterium-Ammonia Exchange on Metal Powders

Metal (mg)	"Memory" Test	Raction Rate (Increase in % H_2 in 10 minutes)	Reaction Order D_2	Reaction Order NH_3	Activation kcal/mole
Iron, 200	<0.5%	12.5 at 245°	1	0	17.5
Ni, 100	<0.5	6 at 229°	0.5-1	0	9.5
W, 100	~0	2 at 413°	1	0	25.4
		6 at 447°			
Mo, 99	~2%	12 at 313°	1	0	12.8
		6.5 at 288°			
Cu, 110a)	~1%	4 at 231°	<1	>0	18.5
Co, 95b)	0	3 at 90°	—	—	—
Ag, 100c)	3%	4.5 at 340°	1	—	—
Zr	~30%	—	—	—	—

Notes: a) Contained some oxide.
b) Prepared by reduction of the oxide. The sample was very active, but lost its activity rapidly.
c) Prepared by reduction of the oxide.

TABLE II
Deuterium-Ammonia Exchange on Metal Wires

Metal (mg)	Diameter (mm)	Temperature 0°C	Activation Energy kcal/mole
Pt, 318	0.29	358—400	14.4
Ni, 156	0.32	360—450	9.9
Pd, 598	0.50	247—304	5.4

REFERENCES

1. FARKAS, 1936, *Trans. Faraday Soc.,* **32**, 416.
2. WEBER and LAIDLER, 1951, *J. Chem. Phys.,* **19**, 1090.
3. SINGLETON, ROBERTS and WINTER, 1951, *Trans. Faraday Soc.,* **47**, 1318.
4. KEMBALL, *Trans. Faraday Soc.,* 1952, **48**, 254.

Papers presented in the Section of Biochemistry

On the Mode of Action of Antibiotics

The experiments with a wild strain and with mutants of *Escherichia coli* had shown that chloramphenicol (chloromycetin) inhibits the growth of this organism by interfering with the biosynthesis of indole from anthranilic acid, probably via an unknown intermediate[1]. An investigation of a number of other antibiotics has led to the following results: *Bacitracin* is completely inactive even in concentrations of 4000 γ/ml; on the other hand *dihydrostreptomycin* inhibits the growth of *E. coli* in concentrations of 0.4 γ/ml, and no reversing agent has yet been found (the following substances have been tested: anthranilic acid, indole, L-tryptophan, DL-phenylalanine, L-tyrosine, and DL-methionine).

Aureomycin and *terramycin* inhibit the growth of *E. coli* by the same mechanism as chloramphenicol: (a) The inhibition is reversed by indole, tryptophan and—to a lesser degree—by phenylalanine and tyrosine, but not by anthranilic acid.

(b) Whilst the inhibition of an indole-less, a tryptophan-less, a phenylalanine-less and a tyrosine-less mutant of *E. coli* is reversed by the corresponding metabolites, that of an anthranilic acid-requiring mutant is not reversed by anthranilic acid.

(c) Both in the wild strain and in the anthranilic acid requiring mutant, the inhibition is reversed by the combination of anthranilic acid, methionine and vitamin B_{12}.

It appears, therefore, that as in the biosynthesis of purines[2,3,4] a C_1 unit is involved in the synthesis of the indole system and that the source of this unit is the S-methyl group of methionine. This, incidentally, would tend to show that in *E. coli* the conversion of anthranilic acid to indole does not involve the elimination of the carboxyl group of the former, as seems to be the case in *Neurospora crassa*[5].

This identity of the mode of action of chloramphenicol, aureomycin and terramycin gives the *biochemical explanation for the known cross-resistance* of bacteria to these three antibiotics. A micro organism, e.g., which has become resistant to chloramphenicol, has also acquired resistance to aureomycin and terramycin[6,7] but not to streptomycin[7]. Also the morphological changes brought about by the acquisition of resistance to streptomycin are different from those caused by the other three antibiotics[8].

The organic chemist expects eventually to find a relationship between the chemical structure of a biologically active substance and its mode of action. It will be difficult to find such an explanation for the identical mode of action of chloramphenicol, a derivative of *threo*-phenylserinol,

and terramycin, a compound of the naphthacene series[9].

ERNST D. BERGMANN
*Scientific Department,
Ministry of Defence*
SARAH SICHER
BENJAMIN E. VOLCANI
*Weizmann Institute of Science,
Rehovot.*

REFERENCES

1. BERGMANN, E. D. and SICHER, S., 1952, *Nature*, **170**, 931.
2. BEN-ISHAI, R., VOLCANI, B. and BERGMANN, E. D., 1951, *Bulletin Res. Council, of Israel*, **1**, No. 1-2, 160.
3. BERGMANN, E. D., VOLCANI, B. E. and BEN-ISHAI, R. 1952, *J. Biol., Chem.*, **194**, 521.
4. BERGMANN, E. D., BEN-ISHAI, R. and VOLCANI, B. E., 1952, *J. Biol. Chem.* **194**, 531.
5. NYC, J. F., MITCHELL, H. K., LEIFER, E. and LAUGHAM, W. H., 1949, *J. Biol. Chem.*, **171**, 783.
6. PANSY, F. E., KHAN, P., PAGANO, F. and DONOVICK, R., 1950, *Proc. Soc. Exp. Biol. Med.*, **75**, 618,
7. HERRELL, W. E., HEILMAN, F. R. and WELLMAN, W. E., 1950, *Ann. N.Y. Acad. Sci.*, **53**, 448,.
8. PULVERTAFT, R. J. V., 1952, *J. Pathol. Bacteriol.*, **64**, I, 75.
9. HOCHSTEIN, F. A., STEPHENS, C. R., CONOVER, L. H., REGNA, P. P., PASTERNACK, R., BRUNINGS, K. J. and WOODWARD, R. B., *J.*, 1952, *Amer. Chem. Soc.*, **74**, 3528

The Action of 5-Fluorotryptophan on Escherichia coli

p-Fluorophenylalanine (I) inhibits the growth of *Escherichia coli* by interference with the utilisation of phenylalanine[1,2,3,4] and acts by the same mechanism on higher animals[5]. It seemed interesting to study analogously the response of bacteria to 5-fluorotryptophan (II)[6]
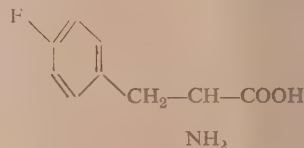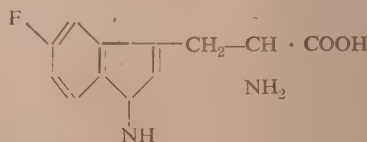
5-Fluorotryptophan is a most active inhibitor of the growth of *E. coli*; already 0.1 γ/ml prevents growth completely. The inhibition is reversed by tryptophan, also by phenylalanine and tyrosine and, on the other hand, by indole, not, however, by anthranilic acid. Furthermore, the in-

hibition of the indole-less, the tryptophan-less, the phenylalanine-less and the tyrosine-less mutant of *E. coli* is reversed by the respective metabolites, whilst that of the anthranilic acid-requiring mutant is not relieved by anthranilic acid. The point of attack of the fluoro-compound is, therefore, the biosynthetic step which leads from anthranilic acid to indole. If the mode of action of 5-fluorotryptophan were analogous to that of *p*-fluorophenylalanine, one would have to expect[1,2] that the most effective reversing agent in the wild strain would be tryptophan and that the latter and 5-fluorotryptophan would show most clearly competitive antagonism in the tryptophan-less mutant.

The analogy between the mode of action of 5-fluorotryptophan and that of the antibiotics aureomycin, terramycin and chloramphenicol[7,8] is thrown into relief even more by the observation that also in the case of the fluoro-compound the inhibition of the anthranilic acid-requiring mutant of *E. coli* is reversed by the ternary system anthranilic acid-methionine-vitamin B_{12}. The high activity of 5-fluorotryptophan is remarkable; whilst 0.3 γ/ml of terramycin, 2.0 γ/ml of chloramphenicol and 1.6 γ/ml of aureomycin cause only a 30—40% reduction of growth, 0.1 γ/ml of the fluoro-compound inhibits growth completely, and it is rather striking that addition of methionine and vitamin B_{12} reverses this complete inhibition to full growth.

The difference in behaviour of the fluoro-compounds (I) and (II) is rather surprising, the more so as also *o*- and *m*-fluorophenylalanine act in the same manner as the *p*-compound[9]. One would expect an inhibitor of anthranilic acid utilisation to be very much more similar to anthranilic acid[10], unless the solution of the problem lies in the structure of an unknown intermediate between anthranilic acid and indole.

ERNST D. BERGMANN
EMILE ESCHINAZI
Scientific Department,
Ministry of Defence
SARAH SICHER
BENJAMIN E. VOLCANI
*Weizmann Institute of Science,*
Rehovot.

REFERENCES

1. BERGMANN, E. D., SICHER, S. and VOLCANI, B. E., 1952, *Biochem. et Biophys. Acta*, 9, 220.
2. BERGMANN, E. D., SICHER, S. and VOLCANI, B. E., 1952, *Biochem. J.*, (in press).
3. ATKINSON, D. E., MELVIN, S. and FOX, S. W., 1951, *Arch. Biochem.*, 31, 20.
4. MOORE, G. I. and MON, I. N., 1949, *Amer. J. Pharm.*, 121, 1.
5. ...., M. D. and LEWIS, J. D., 1951 / *Amer. Chem.*, 198, 41.
6. ...., H. and NIEMANN, C., 1950, *J. Amer. Chem.*, 72, 2296.
7. BERGMANN, E. D. and SICHER, S., 1952, *Nature*, 170, (in press).
8. BERGMANN, E. D., SICHER, S. and VOLCANI, B. E., 1952, *Biochim. et Biophys. Acta*, 2, (in press).
9. ...., 1. D. SICHER, S. and VOLCANI, B. E., 1952, *Arch. Biochem.*, 74, (in press).
10. RYDON, H. N., 1948, *Brit. J. Exp. Path.*, 29, 48.

The Action of α-Methylglutamic Acid on Enzymatic Systems Connected with Glutamine Metabolism

D,L-α-methylglutamic acid markedly inhibits sheep brain glutamotransferase and dog kidney glutaminase. It is known that sheep brain also contains an enzyme system which synthesizes glutamine from ammonia and glutamic acid in the presence of ATP. When in this system glutamic acid is replaced by α-methylglutamic acid, a synthesizing reaction occurs. On the basis of the evidence available, the end product of this reaction appears to be α-methylglutamine. Similar results were obtained with the glutamine synthesizing enzyme system present in pigeon liver extracts.

N. LICHTENSTEIN
Department of Biochemistry,
Hebrew University, Jerusalem,

H. E. ROSS,
P. P. COHEN
Department of Physiological Chemistry
University of Wisconsin,
Madison, Wisconsin.

Enzymatic Hydrolysis of Alkyl Fluoroacetates and Related Esters

The esters of fluoroacetic acid show an optimum in their pS-activity curves, when hydrolyzed either by choline-esterase or an unspecific esterase. In the former case the position as well as the height of the pS optimum is a function of the length of the alkyl chain, but this rule does not hold for liver esterase. Other halogenoacetates behave in a similar way.

Acetylcholine exhibits a pS optimum only during hydrolysis by acetylcholine esterase, but not by liver esterase. Thus two different principles must be involved in the auto-inhibition of ester hydrolysis by choline esterase.

If pK_m (the negative logarithm of the Michaelis-Menten constant) is plotted as a function of pK_a for the systems halogenoacetates-liver esterase, a straight line is obtained, indicating that here the rate of hydrolysis is determined by the electronegativity of the halogen substituent. However, the same graph for choline esterase shows a maximum for chloroacetate. It is concluded that fluoroacetate behaves abnormally towards this enzyme.

F. BERGMANN
E. SHIMONI
Dept. of Pharmacology,
Hebrew University-
Hadassah Medical School,
Jerusalem

The Influence of pH Changes on the Rate of Enzymatic Hydrolysis of Alkyl Halogenoacetates

The pH-activity curves for the hydrolysis of esters by liver esterase are essentially identical for all substrates, whether they contain a halogen substituent or a simple aliphatic acyl residue. The maximum is found at pH 8.

The corresponding curves for choline esterase are identical with the results on liver esterase in the case of ethyl chloro- and bromo-acetate. The curve for acetylcholine deviates from these "standard" substrates mainly on the alkaline side (above pH 8). This is explained by the fact that acetylcholine combines both with the esteratic site and the negative charge of the active surface.

The pH-activity curve of the system fluoro-acetate-choline esterase is abnormal: Between pH 8—9.5, the rate of hydrolysis increases by about 100%. This shows that in addition to the two groups in the esteratic site, G_1 (the nucleophilic component) and G_2 (the electrophilic component), identified in our earlier experiments, a third group G_3 must be present, which combines specifically with fluoroacetate, but with no other substrate.

F. BERGMANN
E. SHIMONI
Dept. of Pharmacology,
Hebrew University-Hadassah Medical School,
Jerusalem

Reduction of Disulfide-bonds of Human Serum Albumin by Thioglycollic Acid

The human serum albumin molecule contains 18 S-S-cysteine bonds[1]. These serve as cross links between different parts of the peptide chain and together with the many amide hydrogen bonds present, stabilize the shape of the molecule. The purpose of the present investigation was to determine the ease of reduction of the human serum albumin disulfide bonds by specific reducing agents such as thioglycollic acid and to evaluate the changes effected in the macromolecule by the reductive cleavage of these cross links.

In most of our experiments a solution of 2.86×10^{-4} M human serum albumin and a 5×10^{-2} M thioglycollic acid was used. The preparation of human serum albumin used had a molecular weight of 69,000 and contained somewhat less than one SH-group per molecule. All reductions were carried out at $0°C$ under nitrogen. The mixture was brought to the desired pH by strong acid or base.

The extent of reduction was calculated from the number of SH-groups acquired by the protein. In some of the experiments, thioglycollic acid was removed from the reaction mixture by a suitable ionic exchanger, in others the protein was purified by precipitation. The number of protein-SH-groups was determined by titration with methylmercuric-nitrate (10^{-3} M), in the presence of guanidine, using sodium nitroprusside as indicator[2]. Cysteine in the hydrolysates of the SH-enriched albumin preparations was determined according to Nakamura and Binkley[3] and found to be equivalent to the number of SH-groups titrated according to the technique given above.

The extent of reduction of the human serum albumin by thioglycollic acid at different pH values
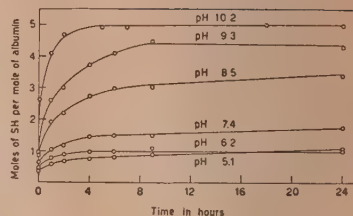
Figure 1

Reduction of human serum albumin (2.86×10^{-4} M) by thioglycollic acid (5×10^{-2} M). Temperature $0°C$.
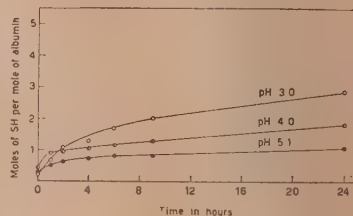
Figure 2

Reduction of human serum albumin (2.8×10^{-4} M) by thioglycollic acid (5×10^{-2} M). Temperature $0°C$.

is given in Figures 1 and 2. In the reported experiments, reduction proceeded to completion during 24 hours. The rate of reduction increases with pH. When the excess of thioglycollic acid is large, as is the case in our experiments, the maximal number of SH-groups acquired by the albumin is set by the pH and is independent of the protein concentration within a wide limit. The number of SH-groups acquired per molecule of human serum albumin on exhaustive reduction is shown in Figure 3 as a function of pH.

In the range of pH 4.2 to pH 7.0, the reduction of human serum albumin leads to a protein with one SH-group per molecule. Mercaptalbumin[2] which differs from normal human serum albumin only in the possession of a single SH-group per molecule is not reduced under these conditions. In the pH range 7 to 10, the number of SH-groups

Figure 3

Number of SH-groups acquired per molecule of human serum albumin, on exhaustive reduction, as a function of pH ($0°C$). Each point represents an average of a number of experimental data.

per molecule increases from 1 to 5; a similar but smaller increase is also found in the pH range 4.2 to 2.

Turbidity and sedimentation measurements proved that no change in molecular weight occurred when the protein was treated with thioglycollic acid in the pH range 4.2 to 7.0, and that an 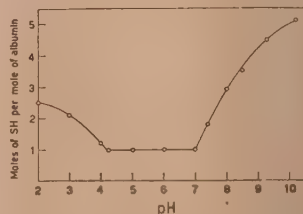increase in the apparent molecular weight, due possibly to partial denaturation, aggregation or polymerization of the SH-enriched protein occurred when the reduction was carried out at pH values higher than 7.0 or lower than 4.2.

The addition of a denaturing agent, guanidine, to the reduction mixtures markedly increased the number of reduced disulfide bonds, the effect being especially marked at the higher pH values. In the presence of 2.3 M guanidine at pH 9.0, for example, the serum albumin acquired 28 SH-groups per molecule in a short time. In these conditions, a large majority of the —S—S— bonds present in the molecule were reduced.

From the experiments described above, it may be concluded that in the pH range 4.2 to 7.0, all the eighteen —S—S— bonds of the molecule are "hidden" in the molecule and cannot be reduced by thioglycollic acid. In the pH range 7.0 to 9.0 the molecule presumably changes its shape reversibly and exposes a small number of —S—S— links which may then be reduced by the reducing agent. At pH values higher than 10, the molecule undergoes an irreversible change which leads to a further increase in the number of exposed disulfide bonds. The maximal exposure of —S—S— groups occurs in the presence of guanidine which causes at alkaline pH a complete "unfolding" of the molecule. Somewhat similar changes probably occur also at pH lower than 4.2. The conclusions drawn are in complete agreement with those derived by Klotz[4] from his studies of the amount of dye bound by serum albumin at different pH values.

It seems that in human serum albumin, and perhaps also in other proteins, a clear distinction should be made between irreversible changes in the protein molecule (mainly referred to as denaturation) and reversible changes leading to a reversible exposure of "hidden" chemical groups.

EPHRAIM KATCHALSKI
Department of Biophysics
Weizmann Institute of Science,
Rehovot

GEORGE S. BENJAMIN
University Laboratory of Physical
Chemistry, Harvard University,
Boston

REFERENCES

1. Brand, E., 1946, *Annals of the New York Academy of Science*, **47**, 187.
2. Hughes, W. L., 1950, *Cold Spring Harbor Symposia*, **14**, 79.
3. Nakamura, K. and Binkley, F., 1947, *J. Biol. Chem.*, **173**, 407.
4. Klotz, I. M., *Trans. Faraday Soc.*, (in press).

A Micromethod for the Estimation of Cellulose and its Use in the Study of the Synthesis of Cellulose by Cell Suspensions of Acetobacter xylinum

A cellulose-poor washed cell suspension of *Acetobacter xylinum* forms cellulose from glucose in a medium of simple composition[1], and thus appears to be uniquely suitable for study of the mechanism of this important reaction. The published procedures for the estimation of small amounts of cellulose are, however, either very tedious or non-specific. A search for a technique which would be very suited to serial determinations of microgram quantities of bacterial cellulose was therefore undertaken by us.

A method whose basis is described below appears to meet these requirements:

a) The cellulose in the resting cell suspension is purified by extraction with suitable aqueous solvents, the removal of the solvent by centrifugation being facilitated when its specific gravity is lowered through an addition of ethyl ether to the fluid.

b) The purified cellulose is esterified and acetolyzed with acetic anhydride in acetic acid, sulfuric acid serving as the catalyst.

c) The acetolyzed product is hydrolyzed quantitatively with sulfuric acid after dilution in water.

d) The reducing power of the neutralized hydrolyzate is measured colourimetrically with Nelson's reagent and Somogy's copper reagent.

The acetolysis of a cellulose is itself rapidly completed and greatly facilitates the subsequent hydrolysis of the glucosidic linkages. Incidentally the same process also affords a convenient means for subdivision of the sample into desired aliquots. The high concentration of salts in the neutralized hydrolyzate does not interfere with the estimation of reducing power with the method specified. Recoveries of reducing sugar from samples of filter-paper cellulose and *xylinum* cellulose corresponded to approximately 95% of the theoretical expectation. Determinations of cellulose quantities in the range 30—1000 micrograms were reproducible within an error range of approximately ± 4%.

The application of the method to measurement of the cellulose-synthesizing activity of cell suspensions of *Acetobacter xylinum* will be illustrated and some general properties of this system will be described.

MICHAEL SCHRAM
SHLOMO HESTRIN,
Department of Microbiological
Chemistry,
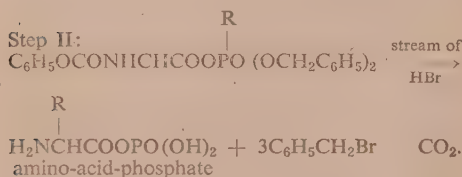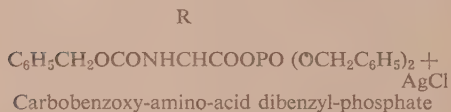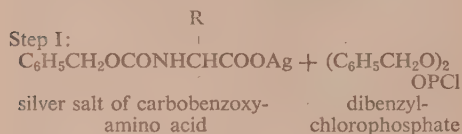*Hebrew University—Hadassah*
Medical School, Jerusalem

REFERENCE

1. Hestrin, S., Aschner, M. and Mager, J., 1947, *Nature* **159**, 64.

Phosphate-anhydrides of Amino Acids

The possibility of formation of phosphate anhydrides of amino acids, as intermediary, energy-rich substances in the synthesis of proteins in the living body, has been discussed by several research workers[1,2,3,4,5].

·Although many efforts ˙have been made, the phosphate-anhydrides of free amino acids have not been isolated, but intermediary substances have been obtained[6,7,8,9,10,11,12,13].

In this laboratory, many experiments have been made in order to get these interesting substances, and lately we succeeded in the preparation of mixed anhydrides of amino acids and phosphoric acid in the free state, in the following way:

Step I:

$$R$$
$$C_6H_5CH_2OCONHCHCOOAg + (C_6H_5CH_2O)_2$$
$$OPCl$$

silver salt of carbobenzoxy-   dibenzyl-
     amino acid   chlorophosphate

$$R$$
$$C_6H_5CH_2OCONHCHCOOPO\ (OCH_2C_6H_5)_2 +$$
$$AgCl$$

Carbobenzoxy-amino-acid dibenzyl-phosphate

Step II:

$$R$$
$$C_6H_5OCONHCHCOOPO\ (OCH_2C_6H_5)_2 \xrightarrow[HBr]{\text{stream of}}$$

$$R$$
$$H_2NCHCOOPO(OH)_2 + 3C_6H_5CH_2Br \qquad CO_2.$$
amino-acid-phosphate

In this way the phosphates of glycine, alanine and leucine were prepared. The phosphates are highly hygroscopic and decompose easily in aqueous solutions. Interesting is the ease with which the phosphoric anhydrides of amino acids couple with free amino acids to give peptides. They also polymerize in solution to give high polypeptides. This property makes plausible the possibility that phosphates of this type are the precursors of protein formation in living organism.

Glycine-phosphate

. To the solution of dibenzyl chlorphosphate[8] in carbon tetrachloride, a little more than an equivalent of the silver salt of carbobenzoxyglycine[14] is added. The mass is agitated for 2 hours at room temperature by a stream of nitrogen. The mixture is left overnight, in order that the colloidal AgCl should deposit. The AgCl is filtered off and the carbon tetrachloride distilled off under reduced pressure. The remaining carbobenzoxy glycine dibenzyl-phosphate is an heavy oil. which after being dried under a pressure of 1/10 mm, gives the following analysis: (Calcd. for $C_{24}H_{24}O_7NP$: C,61.4; H,5.1; N,2.9; P,6.6. Found: C,62.0; H,5.1; N,2.7; P,6.6).

In order to prepare the free glycine phosphate, a stream of HBr was passed through a 10% solution of carbobenzoxy-glycine dibenzyl-phosphate in carbon tetrachloride[15], care being taken to prevent contact with water vapour. The solution gets warm during the reaction with HBr. At saturation with the gas, glycine-phosphate precipitates as a heavy oil. The oil is washed several times with absolute ether, and dried at room temperature at a pressure of 10^{-4} mm. The glycine phosphate could not be obtained at a purity higher than 70%.

In absolute alcohol the glycine phosphate gives ethyl glycinate, which after ether is added, precipitates as the phosphate salt. M.p. 173°. (*Anal.* Calcd. for $C_4H_{12}O_6NP$: N,7.0; P,15.4; CH_3CH_2O,22.3. Found: N,6.9; P,15.4; CH_3CH_2O, 21.6).

It is also easily obtained as the picrate, m.p. 157°.

We studied the hydrolysis of glycine-phosphate in water solutions at 10°C, by potentiometric titrations. It was found that at pH 7.2 the hydrolysis proceeds within 60 minutes, more quickly at pH 7.5 and is instantaneous at pH 8.5.

When glycine-phosphate is shaken in a buffer solution of pH 7.2 at 10°C, it partially decomposes and is partially transformed into polyglycine which precipitates. The ratio of nitrogen found by the Dumas method for this polyglycine to that found by the van Slyke method is 10/1.

In the solution there remain still the lower peptides, which are detected by paper chromatography.

Alanine-phosphate

The alanine-phosphate was prepared by the same method as the glycine-phosphate. It too is an oil which could not be purified to a better extent than 70%.

In a buffer solution the alanine-phosphate gives polyalanine. Here all the fractions remain in solution. The ratio between Dumas-nitrogen and van Slyke-nitrogen is 4/1.

While drying in vacuum, some of the alanine phosphate gives already polyalanine, while part of it passes into the ring form:

$$HN-CH-CH_3$$
$$HO-P\quad C=O$$
$$O$$
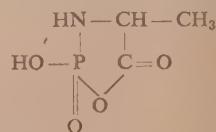
Leucine-phosphate

The carbobenzoxy-leucine dibenzyl-phosphate is obtained in the same way as the carbobenzoxy-glycine dibenzyl-phosphate. It is an oil. (*Anal.* Calcd. for $C_{28}H_{32}O_7NP$: C,63.9; H,6.1; N,2.7; P,5.9. Found: C,64.4; H,6.5; N,2.5; P,6.2).

With hydrobromic acid, the phosphate anhydride of leucine precipitates as the HBr salt. It is a white, crystalline substance, m.p.

H185°.*Anal*. Calcd. for $C_6H_{15}O_5NPBr$: C,24.6; H,5.1 ; N,4.8 ; P,10.6 ; Br,27.4. Found : C,24.9; (,5.3; N,4.9; P,11.0; Br,26.7).

AHARON KATCHALSKY
MELLA PAECHT
Deparment of Polymer Research,
Weizmann Institute of Science,
Rehovot

REFERENCES

1. LIPMANN, F., *Adv. in Enzym.*, Vol. I, p. 154.
2. BORSOOK, H. and DUBNOFF, J. W., 1947, *J. Biol. Chem.*, 168, 397.
3. COHEN, P. P. and MC.GILVERY, R. W., 1949, *J. Biol. Chem.*, 171, 121.
4. SPECK, J. F., 1949, *J. Biol. Chem.*, 179, 1405.
5. SCHULTZ, G. V., 1950, *Naturwissenschaften*, 37, 196.
6. ATHERTON, F. R., OPENSHAW, H. T. and TODD, A. R., 1945, *J. Chem. Soc.*, 382.
7. ATHERTON, F. R., OPENSHAW, H. T. and TODD, A. R., 1945, *J. Chem. Soc.*, 660.
8. ATHERTON, F. R., HOWARD, H. T. and TODD, A.R., 1948, *J. Chem. Soc.*, 1106.
9. CHANTRENNE, H., 1949, *Nature*, 164, 576.
10. CHANTRENNE, H., 1947, *Nature*, 160, 603.
11. CHANTRENNE, H., 1948, *Biochim. et Biophys. acta*, 2, 286.
12. CHANTRENNE, H., 1950, *Biochim. et Biophys. acta*, 4, 484.
13. SHEEHAN, J. and FRANK, V. S., 1950, *J. Am. Chem. Soc.*, 72. 1312.
14. BERGMANN, M. and ZERVAS, L., 1932, *Ber.*, 65, 1192.
15. BEN ISHAI, D. and BERGER, A., *J. Org. Chem*, in press.

Various Plants as Vitamin A Suppliers for Chicks

In order to investigate the efficiency of green plants as vitamin A suppliers instead of cod liver oil, several feeding experiments were carried out with various groups of chicks, 100 chicks in each group. In addition to the usual feeds, each group received a supplementary source of vitamin A as follows : clover, horse beans, green oats, cow peas, corn leaves, sweet potato leaves. The experiments were carried out in different seasons, according to the availability of these plants. In each season one group of chicks was taken as a control and cod liver oil of about 500 units vitamin A was added per 100 g feed. Prior to each experiment the carotene contents of the green fodder was ascertained and the daily supplementary ration (containing 500 units vitamin A per 100 g feed) thus calculated.

The gain in weight of the chicks and the contents of vitamin A in the liver were taken as measure of the activity of the various plants as vitamin A suppliers. Every week the chicks were weighed and in the case of a few the livers were analyzed. Figures for the end of the experiment (the 8th week) are shown below.

It seems that there was no difference in the gain of weight due to the different sources of vitamin A, but the different sources of vitamin A were clearly indicated according to their respective capacities to bring about concentrations of vitamin A in the liver. Several plants such as corn leaves were responsible for doubling the concentration of vitamin A in the liver·as compared with cod liver oil. On the other hand the analyses of livers of

Source of vitamin A	Corn leaves	Green oats	Cow peas	Sweet potato leaves	Clover	Horse beans
The relative weight of the chicks in % of cod liver oil group as 100%	100	98	101	100	89	96
Mean content of vitamin A in the liver in % of cod liver oil group as 100%	208	182	170	40	20	2

chicks which were supplied with clover or horse beans indicated almost no vitamin A content.

Various experiments were carried out to investigate the reasons for the variation in the efficiency of plants as vitamin A suppliers.

1) Digestion experiments with individual chicks showed that the chicks digested about 40% of the carotene in the plant. The digestibility of the carotene does not vary with the species of the plant.

2) According to Kelley and Day[1] the carotene activity can be inhibited by the xanthophyll present in the feed; examinations of the xanthophyll showed that the ratio xantophyll: carotene is the same in all the plants which were used as sources of vitamin A.

3) It was found by Deuel and Greenberg[2] that the stereo-isomers of the β-carotene, present in plants, such as Neo-β-carotene-B, all - trans -β-carotene and Neo-β-carotene-U, have different biological activities. The separation of the isomers was carried out in the above mentioned plants by Bickof's chromatographic method[3] and it was proved that the various isomers were equally distributed in all these plants.

Lepkovsky et al.[4] showed that alfalfa meal contains substances which inhibit the growth of chicks. According to his investigations these substances belong to the saponins. It is considered likely that the content of these inhibitors varies in different plants and work is being continued on these lines.

A. BONDI
E. ASKARELLI
Agricultural Research Station
and Faculty of Agriculture,
Hebrew University, Rehovot

REFERENCES

1. KELLEY and DAY, 1950 *J. Nutrition*, 40, 159.
2. DEUEL and GREENBERG. 1949 *Arch. Bioch.*, 23, 159.
3. BICKOF, 1949 *J. Assoc. Off. Agric. Chem.*, 32, 766.
4. LEPKOVSKY et al., 1950 *Poultry Science*, 29, 208.

Intermediate Products formed on Proteolytic Digestion of Protein Feeds *

In order to investigate the difference between the protein digests of animal and plant protein feeds, these were digested by pancreatin, by fresh carp pancreatic juice (in phosphate buffer pH 8.2), by pepsin (in HCl pH 1.5) and by papain (HCN-activated in phosphate buffer pH 5 and not activated in phosphate buffer pH 7.5).

The defatted feeds examined were: fish meal, meat meal, soy bean oil meal, sesame oil meal and peanuts.

The following experiments were carried out:

A. The terminal NH_2 and COOH groups liberated after 1—5 days of enzymatic digestion were determined (van Slyke, Pope-Stevens and Sorensen). No significant difference in rate of liberation between animal and plant protein feeds was found. However, it should be pointed out that the ratio NH_2 : COOH in the proteolyszates differed generally from 1, it was constant for each enzyme and did not depend upon the substrate. This ratio was 1.2 for pancreatin, 1.25 for papain (HCN-activated in phosphate buffer pH 5) 0.95 for papain (in phosphate buffer pH 7.5), and 0.8 for pepsin.

B. The following principal difference between plant and animal protein feeds was observed: An addition of 5% $CCl_3.COOH$ solution to previously acidified (HCl) pancreatic digests (or to papain digests pH 7.5) of plant protein feeds, yielded considerable precipitates containing 20—30% of the nitrogen of the original substance. Digests of animal protein feeds treated in a similar manner, yielded no precipitates. This difference between the groups of feeds was not due to different rates of digestion, since on addition of $CCl_3.COOH$ to animal protein digests, even after short times of digestion (1/2—4 hours) no precipitate was obtained.

However, considerable precipitates were obtained on addition of $CCl_3.COOH$ to plant protein feeds treated with phosphate buffer (pH 7.5, 8.2) only. In similar digestions with both kinds of feeds carried out with pepsin (pH 1.5) and with papain (HCN-activated pH 5), no precipitates were obtained on the addition of $CCl_3.COOH$.

These facts lead to the conclusion that plant protein feeds contain a protein fraction which is soluble in solutions of pH ~ 8 and is not attacked or slightly attacked by the pancreatic enzymes. This fraction is probably typical of plant proteins, and is not due to the linkage between the latter and the carbohydrates present in the plant feeds as similar results have been obtained on the addition of $CCl_3.COOH$ to pancreatic digests of purified plant proteins (from peanuts for instance).

C. Precipitates obtained on the addition of $CCl_3.COOH$ to several pancreatic plant protein digests were isolated and purified. These products resembled each other very closely in qualitative and quantitative chemical reactions. Paper chromatography of the various acid hydrolysates gave almost identical spots of the same Rf.

Attention should be paid to the possibility that the existence of this protein fraction, which is more stable towards enzymes, is one of the reasons for the difference in nutritional value between plant and animal proteins.

A. BONDI
Y. BIRK *
*Agricultural Research Station,
Rehovot*

Polycysteine

In view of the unique role played by cysteine in protein chemistry, polycysteine was prepared. On the one hand the S—S bonds of its oxidized form are held to be responsible for stabilizing the specific geometrical structure of protein molecules, and on the other hand, the quantitative determination of SH groups is a powerful tool for the investigation of structural changes in proteins, especially during denaturation.

The method adopted was the N-carboxy-anhydride synthesis. The benzyl group, commonly employed to protect the sulfhydryl during peptide synthesis, proved unsuitable in this case, as it could not be removed after polymerization. The monomer finally employed was S-carbobenzoxy-N-carboxy-cysteine anhydride, which was polymerized by heating in benzene with traces of dimethylamine as a catalyst. Poly-S-carbobenzoxy-cysteine could be converted into polycysteine by treatment with sodium in liquid ammonia.

Polycysteine is soluble in water at pH 9 and above, and in ethanolamine. It is sparingly soluble in dimethyl-formamide and insoluble in glacial acetic acid.

The sulfhydryl groups of polycysteine can be quantitatively determined by titration of the iodide ion liberated by interaction with iodoacetate in aqueous alkali. Other methods commonly used for this purpose (iodine, p-chloromercuric benzoate, methyl-mercuric nitrate, the last two with nitroprusside as indicator) gave low values (85%, 68%, and 47%, respectively). Guanidine hydrobromide (5 molar) had no influence on the titration values. However p-chloromercuric benzoicacid reacts quantitatively with the sulfhydryl groups, as can be shown by the isolation of the reaction product and quantitative determination of the carboxyl groups and the nitrogen content.

Potentiometric titration of polycysteine with aqueous alkali fails owing to the low dissociation constant of the SH-group. Polycysteine can, however, be titrated to a distinct end-point in ethanolamine solution with sodium β-amino-ethoxide

using an antimony electrode in the presence of a current of air.

Polycysteine and poly-carbobenzoxy-cysteine are converted into polycysteic acid by oxidation with performic acid in aqueous formic acid. Amino nitrogen determination showed that little if any degradation occurs during oxidation. On hydrolysis, all the nitrogen appeared as amino-nitrogen. Polycysteic acid is extremely soluble in water as are its Na, NH$_4$, Ba, Pb, Ag and Cu salts. An insoluble precipitate is obtained with polyly-sine.

EPHRAIM KATCHALSKI
ARIE BERGER
Department of Biophysics,
Weizmann Institute of Science,
Rehovot

Polytyrosine

As a continuation of the study of the physical and biological properties of poly-α-amino acids[1], we have synthesised polytyrosine and studied some of its properties.

N,O-Dicarbobenzoxy-*L*-tyrosine (I) was prepared from *L*-tyrosine and benzyl chloroformate, and *O*-carbobenzoxytyrosine-*N*-carboxyanhydride (II) from (I). (II) was polymerized with the evolution of carbon dioxide, either in benzene solution at 80° or in bulk in a high vacuum. The degree of polymerization of poly-*O*-carbobenzoxytyrosine (III) was determined by end-group amino nitrogen analysis (van Slyke). Polymers with an average chain-length of 20 to 60 residues were obtained. Polytyrosine (IV) was prepared from (III) by one of the following two methods: shaking with 2N NaOH or shaking with a saturated solution of hydrogen bromide in glacial acetic acid[2]. The value of amino nitrogen of (IV) proved in each case that none of the peptide bonds was attacked during the removal of the carbobenzoxy group.
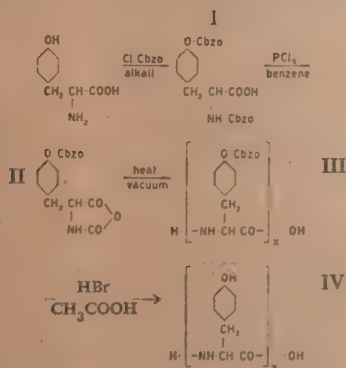
The structure of (IV) was proven by combustion analysis and by total hydrolysis which gave a quantitative yield of tyrosine as shown by amino-nitrogen and ninhydrin-carbon dioxide analysis,

Folin phenol analysis and absorption at 2930A. The structure of (IV) was also supported by the potentiometric titration of the polymer with sodium methoxide in butylamine[3], using an antimony electrode against a glass electrode. This showed that the polymer contains the theoretical number of phenolic groups.

(IV) being a weak acid, is soluble in alkali and precipitated by acids. It is easily soluble in ethyl alcohol and can be precipitated therefrom by ether or water. It is also soluble in glacial acetic acid, dimethyl-formamide and nitrobenzene and is very soluble in organic bases like butylamine, ethanolamine, pyridine or aniline.

Polytyrosine can be iodinated under conditions similar to those for the iodination of proteins and, because of the presence of phenolic groups, it can be coupled with diazonium salts of aromatic amines, producing polymeric dyes. Derivatives of diazonium salts of *p*-bromoaniline and *p*-nitroaniline were prepared yielding poly-3-(*p*-nitrobenzeneazo)-tyrosine (V) and poly-3-(*p*-
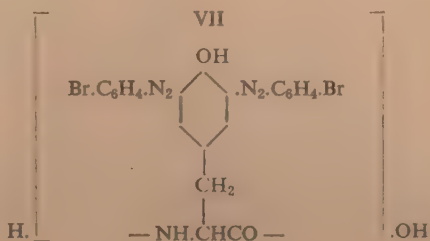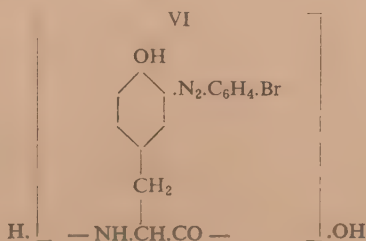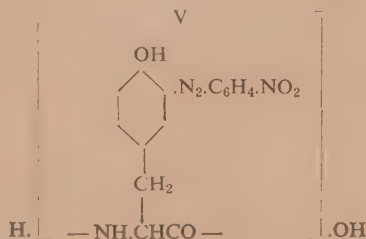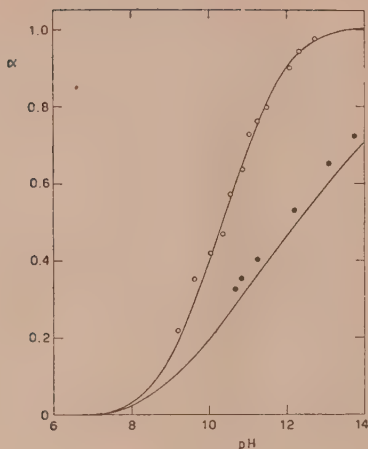
Figure 1

Dependence of the degree of ionization α of polytyrosine on pH. The points represent experimental values. ◯ in the presence of 0.2 M NaCl. ● zero ionic strength. The full curves were drawn from values calculated from the titration equation mentioned in the text.

Figure 2

Dependence of the degree of ionization α of polytyrosine on the ionic strength. The points represent exprimental values at pH 11.5. The full curve was drawn from values calculated from the titration equation mentioned in the text.

bromobenzeneazo)-tyrosine (VI), as well as poly-3,5-bis-(p-bromobenzeneazo)-tyrosine (VII). (V) is soluble in alkali giving a deep violet solution which yields a reddish precipitate on acidification. (VI) and (VII) are insoluble both in alkali and in 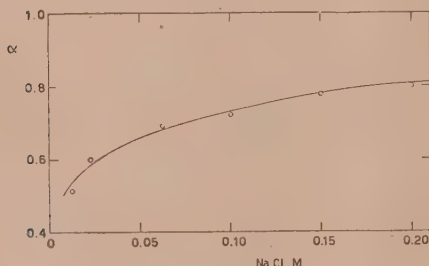acid. They dissolve in dioxane and chloroform (yellow-green solutions) or in alcoholic alkali and organic bases such as butylamine, pyridine and aniline (deep red solutions).

In view of the considerable interest in the absorption of proteins, in the range 2200 A—3200 A[4] we have studied the absorption spectrum of polytyrosine under different conditions. It was found that two absorption peaks ($\lambda = 2410$ A and $\lambda = 2930$ A) appear at strongly alkaline pH's. It is apparent that these two peaks correspond to the absorption spectrum of the phenolate ion of polytyrosine. At acid pH's, the absorption at these two wave lengths is very slight. From the molar extinction coefficients at the two absorption peaks, the degree of ionization, a, of the polymer at different pH's can be calculated. In Figure 1 the spectrophometric titration of polytyrosine under defined conditions is given and in Figure 2 the dependence of the degree of ionization at constant pH on the ionic strength.

From the spectrophotometric titrations it appears that the relation

$$pH = pK_o + 0.868 \; w \; a \; m - \log\frac{1-\alpha}{a}$$

known from the potentiometric titrations of the proteins, is valid also in our case[5]. pK_o is the intrinsic dissociation constant (we have assumed the value of 9.50, close to the value assumed by Tanford[6]), w the electrostatic interaction factor[5], which is easily computed if the dimensions of the molecule and the Debye $\varkappa$ are known; m is the number of phenolic groups in the molecule. We have assumed a radius of 16 A for a 30-mer.

The fact that the aforementioned equation is valid, shows that the absorption of polytyrosine is the absorption of a polymer which contains a great number of phenolic groups, as in the case of proteins. It is too early to decide if the shape of the polytyrosine molecule remains constant during ionization, like that of proteins, or if it changes its shape with increasing degree of ionization.

The details of this investigation will be published elsewhere.

EPHRAIM KATCHALSKI
MICHAEL SELA*
*Department of Biophysics.
Weizmann Institute of Science
Rehovot*

REFERENCES

1. KATCHALSKI, E., 1951, *Adv. in Protein Chem.*, **6**, 123.
2. BEN ISHAI, D. and BERGER, A., 1952, *J. Org. Chem.*, in press.
3. FRITZ, J. S. and LISICKI, N. M., 1951, *Anal. Chem.*, **23**, 589.
4. BEAVEN, G. H. and HOLIDAY, E. R., 1952, *Adv. in Protein Chem.*, **7**, 320.
5. COHN, E. J. and EDSALL, J. T., 1943, *Proteins, Aminoacids and Peptides*.
6. TANFORD, C. and ROBERTS, Jr., G. L., 1952, *J. Amer. Chem. Soc.*, **74**, 2509.

The Reaction Between Aldoses and Basic Polyamino acids or proteins

The reaction of amino acids, proteins and other nitrogen compounds with carbohydrates is fundamental to a wide variety of phenomena, many of which play an important role both in biological processes as well as in the storage and processing of foods. Although the reaction has been studied extensively, its mechanism in solution under mild conditions has not been elucidated.

In order to ascertain the mechanism of the reaction between aldoses and proteins, the kinetics of a simpler system, i.e. aldoses and amino acids, were studied. Because of the lability of the reaction products, the N-glycosides, an experimental method which does not cause interference in the reaction-course was developed. The method is based on the fact that on mixing aldoses with amino acids in solution, a drop in the pH occurs, eqilibrium being reached only after some time. From the change of the quantity b of the alkali addition necessary to maintain the pH constant, the reaction rate constants and the equilibrium constants may be calculated. The reaction scheme is as follows:

$$RH \; \overset{K_A}{\rightleftharpoons} \; R^- + H^+$$

$$+G$$

$$k' \updownarrow k''$$

$$RG^-$$
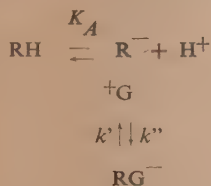
Here RH denotes the amino acid (e. g. $^+NH_3$-CH_2COO^-), R^- its negative ion (e.g. NH_2CH_2-COO^-) and G the aldose. K_A is the acid dissociation constant of the amino group. The equilibrium constant in the reaction is

$$L = \frac{RG^-}{R \cdot G} = \frac{k'}{k''}$$

The reaction rate constants are calculated from the alkali added b with the aid of the following equation:

$$k' = \frac{1}{t} \cdot \frac{L}{(1+LG)} \cdot \frac{b_\infty}{b_0} \ln \frac{b_\infty - b_0}{b_\infty - b}$$

t being time; b_0—alkali added at t, the start of the reaction ($t = 0$); b—alkali addition at time t, and b_∞, total alkali added at equilibrium, ($t = \infty$).

Graphs of $\ln \frac{b_\infty - b_0}{b_\infty - b}$ versus t gives straight

lines, as required by the equation.

The reaction rate constant k' is pH-dependent, decreasing with increasing pH. It also varies with the nature of the amino acid and the aldose.

A detailed mechanism was developed in order to explain these results. According to this mechanism, the constant k' may be resolved into pH independent constants k_α and k_β, when

$$k' = \frac{1}{k_\alpha + k_\beta \; OH}$$

Furthermore, the constant k_β may be deduced from the structure of the amino acids and aldoses. The rate of the primary attack of the acid on the sugar increases with the basicity of the amino component, in accordance with the Hammett relation. Various aldoses react with rates depending on the stability of the pyranose ring.

Further work has shown that the same potentiometric technique is applicable to the study of the reaction between aldoses and basic polyamino acids, e.g. polylysine. The general scheme of the reaction is identical in both cases. However, since polylysine is a polyelectrolyte, its acid dissociation follows an equation, which includes a correction for the electric potential ψ

$$pH = pK_o - \log \frac{\beta}{1-\beta} - 0.4343 \frac{\varepsilon\psi}{kT}$$

(β is the degree of ionization of the polymeric base, ε the charge of the electron).

A detailed calculation shows that the reaction rate constant k' of the reaction may be computed from the kinetic equation characteristic to reactions of polyelectrolytes.

$$k' = \frac{H}{K_o \, Gt} \left[Ei \, (-x_0) - Ei \, (-x) \right]$$

H — hydrogen ion concentration

K_o — intrinsic acid dissociation constant of the basic polymer

G — aldose concentration

t — time

$Ei \, (-x)$ are exponential integrals of the form:

$$-Ei \, (-x) = \int_x^\infty \frac{e^{-u}}{u} \, du$$

$$x = \frac{\varepsilon\psi}{kT} \left[1 - \frac{\triangle b}{c_m \beta} \right]$$

$\triangle b$— alkali addition, c_m— monomolar concentration of the polymer. The rate constant k' of the glucose-polylysine reaction depends only slightly on the pH, a result which is in agreement with studies of the glucose-δ-amino valeric acid reaction. The equilibrium constant L is of the same order of magnitude as for amino acids.

Preliminary studies of the kinetics of aldose-protein interaction have shown that a drop in the pH also occurs on the addition of the aldose, and alkali must be added in order to keep the pH constant. The amount of alkali increases with time, until equilibrium is attained. The theory of this case is now being worked out.

A detailed report will be published elsewhere.

AHARON KATCHALSKY
*Weizmann Institute of Science,
Rehovot.*

NATHAN SHARON*
*Dairy Research Laboratory,
Agricultural Research Station,
Rehovot.*

* From a thesis to be submitted to the Hebrew University, Jerusalem, by Nathan Sharon in partial fulfilment of the requirements for the Ph.D. degree.

Determination of Small Quantities of Amino Acids by the Ninhydrin Reaction

The intensity of the blue colour produced by the interaction of amino acids and ninhydrin, is dependent on the following factors:

1) The structure of the amino acid. In all instances, there is obtained the same colour with a maximum at 5700 A, but its intensity varies with the amino acid. The longer the carbon chain, the lower the intensity; the terminal basic group in lysine, ornithine and arginine is without influence; and the aromatic radicals of phenylalanine, tyrosine and tryptophan reduce the intensity most pronouncedly. Chloride ions make the maximum at 5700 A disappear, and in high concentrations even suppress the colour reaction completely.

2) The ninhydrin reaction is of first order. The aldehyde which is formed simultaneously from the amino acid, reacts with the coloured substance; however, this secondary reaction is inhibited by reducing and by oxidising agents.

3) A method for the determination of small quantities of amino acids (0.2—10 μg) is described, as they appear in chromatograms. The amino acid is extracted from the paper by means of *iso*butyl alcohol and is estimated by the ninhydrin reaction directly in the same solvent.

H. MEYER
R. SCHLESINGER
M. BANAI
*Department of Biological and Colloidal Chemistry,
The Hebrew University, Jerusalem*

On the Synthesis of Vitamin C in the Rat

The intoxication of rats by barbituric acid derivatives leads to urinary excretion of vitamin C. Assuming that the effect is one of enhanced synthesis of the vitamin, we investigated various organs of the rat (*in vitro*) in presence of luminal. The amount of vitamin C in the organs decreases by incubation at 37° during three hours; when luminal is added, an effect is observed in the intestines and the brain where the amount of vitamin C remains constant or even increases during incubation. The conclusion which offers itself is that these two organs are likely sites of the of biosynthesis vitamin C.

H. MEYER
B. EPSTEIN
*Department of Biological and Colloidal Chemistry,
The Hebrew University, Jerusalem*

NEWS AND VIEWS

SYMPOSIUM ON BASIC MEDICAL RESEARCH

A three-day Symposium on Basic Medical Research was held under the auspices of the Hebrew University-Hadassah Medical School in Jerusalem on June 9—11, 1952 with the participation of a Scientific Mission composed of eight eminent scientists from abroad. The Israel participants were from the staff of the Hebrew University-Hadassah Medical School.

Those of the visiting scientists who were members of the American Medical Advisory Board, took part in the Workshop Conference of the Medical School's Board of Management which preceded the Symposium, and most of them took part in laying the cornerstone for the new Hebrew University-Hadassah Medical Centre, for which ground was broken on June 5.

The Organizing Committee for the Symposium was composed of two members in New York (Professors Israel S. Wechsler and David Nachmansohn of Columbia University) and two in Jerusalem (Dr. Alexander Geiger and Dr. Shlomo Hestrin of the Hebrew University-Hadassah Medical School).

The Dean of the Medical School, Prof. A. Dostrovsky, welcomed the Scientific Mission and Prof. Nachmansohn responded on its behalf. Six sessions were held under the chairmanship of Prof. A. Fodor, Prof. B. Zondek, Dr. S. Hestrin, Prof. E. B. Chain, Dr. A. Geiger and Prof. L. Halperin, respectively. The following are summaries of the papers read.

Prof. Louis F. Fieser, *Sheldon Emery Professor of Organic Chemistry at Harvard University*, gave a lecture entitled, "Cortisone, Cholesterol, and Cancer".

Sterols are crystalline, solid alcohols that occur in both animal and plant organisms. The characteristic sterol of animals is cholesterol, $C_{27}H_{46}O$; this water-insoluble lipid is transported in the blood in the form of a specific lipoprotein, a conjugate of the lipid with a globular protein. It is present in all tissues of the body; the total cholesterol amounts to about 1 mg per gram of body weight (of which about 75% is water). Plants synthesize a variety of sterols having one or two carbon atoms more than cholesterol, i.e., C_{28} and C_{29}. Plant sterols of the diet are not the precursors of cholesterol; indeed they are not even absorbed through the intestinal wall. The animal organism can synthesize its own cholesterol, and can do so very rapidly from such a simple carbon source as

sodium acetate. Studies with radioactive acetate have shown that synthesis of the elaborate molecule is effected in the course of a few minutes.

Cholesterol was discovered in 1770. It constitutes some 60—80% of gall stones and can be isolated very easily from this readily available source, and it has been the subject of extensive studies extending for over a century and a half. Investigations aimed at elucidation of the structure were initiated by Adolf Windaus about 1903 and extended by Wieland and others until the problem was finally solved in 1932, when the arrangement of the 27 carbon atoms into a network of 4 rings was established experimentally.

But what is the function of cholesterol? What useful purpose does it have? Why is so much of it required? These questions have not been answered. We still do not know. An indirect function is perhaps indicated by the existence of a series of sterol-like, "steroid" vitamins and hormones that are related to cholesterol and that may possibly be derived from it. The first of these to be recognized is the antirachitic factor, vitamin D_3, discovered by the Windaus group at Goettingen. Vitamin D_3, which is capable, in doses of a few micrograms, of curing the deficiency disease that causes softening of the bones in children, is formed on irradiation of 7-dehydrocholesterol, which differs from cholesterol in having two less hydrogen atoms. The D-factor was recognized as a sterol in the period 1925—1930, and elucidation of structure and synthesis were completed by 1935.

The first known steroid sex hormone, estrone, was isolated in 1929. By 1935 a whole array of male and female sex hormones had been isolated and produced synthetically; it is now recognized that the principal true hormones of the gonads are testosterone, estradiol, and progesterone. The steroids secreted in the cortex of the adrenal gland were investigated chemically in the period 1935—1939 and no less than 27 individual steroids were isolated from this source. Only six of these proved to have physiological actions associated specifically with the adrenal gland. Only one of the six, desoxycorticosterone, was readily enough available by synthesis to permit early exploration of its physiological actions, and it found only limited use in therapy. The hormone now known as cortisone is obtainable from glands only with great difficulty and in minute amounts: 500 mg from 1000 lbs of hog or beef adrenals. Research on the synthetic production was initiated in the U.S.A.

in 1943 as the result of unfounded rumours that the Luftwaffe had found some use for cortical hormones, possibly to increase resistance of pilots to fatigue in sustained flight. Synthesis from a bile acid in an elaborate 36-step process was finally achieved in 1947, and Merck and Co., at great effort and expense, produced a hundred or so grams for exploratory biological and medical studies. Most of it went to the Mayo Clinic, in Rochester, Minnesota, where chemist E. C. Kendall and clinician P. E. Hench tested its effect on patients suffering from rheumatoid arthritis. Dramatic success in alleviation of excrutiatingly painful symptoms was reported in April 1949, and cortisone soon became recognized as a new "wonder drug." Merck achieved the difficult feat of adapting the 36-step synthesis to commercial production at an initial price of $200 per gram, and developmental work in the next two years reduced the price to about $20 per gram. But the dosage requirement is excessively high— 100 mg per day. And the antiarthritic hormone is alleviative and not curative, and hence administration has to be continued indefinitely. The initial estimate was that some 7 million arthritic patients in the U.S.A. alone might require cortisone. It soon became clear that the process utilizing bile acids could not supply the huge amount of drug likely to be required because of limitations in the amount of animal bile available as starting material.

How to produce cortisone from some more abundant source? It seemed to me that the best prospect of a solution would lie in discovery of a way of utilizing available sterols: cholesterol, ergosterol from yeast, and diosgenin from a Mexican dioscorea. The difficulty lay in introduction of oxygen at C_{11} into a ring containing no functional groups permitting chemical manipulation. My research group eventually found a route involving oxidation of a 7,8;9,11-diene and we published a first Communication in May, 1951. A parallel Communication from Merck dated just one day later announced independent discovery of an almost identical method. Further similar routes through 7,8;9,11-dienes were soon reported by Syntex (July), by Jeger's group at Zurich (October), and by Spring's group at Glasgow.

That all of these new routes from sterols to cortisone proceeded through oxidation of a 7,8; 9,11-diene was of particular interest to me in connection with a research on the possible existence of a steroid carcinogen derived from cholesterol by oxidation. An extensive study of the chemical oxidation of cholesterol led me to suspect the presence of one or more companion substances. I eventually succeeded in isolating from spinal cord cholesterol a companion that I call lathosterol (*latho-*, undetected). The substance differs from cholesterol in being very much more sensitive to oxidation. The initial product of oxidation is a 5,7;9,11-diene of just the type that had been found useful for conversion to an 11-oxygenated sterol intermediate to cortisone. One can easily see how lathosterol, present in tissue cholesterol, could become oxidized to an 11-ketosteroid having an important and unique structural characteristic in common with the physiologically potent cortisone. Can such a substance function as a carcinogen? Tests are in progress. Lathosterol is present in cholesterol of various tissues in amounts of from 0.4% to 3%.

"Cholesterol" appears to contain still another companion, tentatively designated thaptosterol (*thapto-*, buried). I have not yet isolated the substance itself but have obtained an oxidation product, "Ketone 104", from a number of sources. Experiments with cholesterol labelled with radioactive carbon are now in progress. When C_{14}-sodium acetate is injected into a rat and the animal is worked up four hours later, the total sterol is found to possess considerable radioactivity. Fractionation shows that the cholesterol, cholestanol, and thaptosterol all have about the same activity, whereas the lathosterol is four times as active. The result suggests that lathosterol is a key metabolite, involved in several paths of biosynthesis."

Dr. Isidore Berenblum *of the Department of Experimental Biology, Weizmann Institute of Science, Rehovot*, lectured on "Cocarcinogenesis".

The isolation and identification of 3:4-benzpyrene from coal-tar led to the discovery of a wide range of synthetic polycyclic hydrocarbons capable of inducing tumours at the site of application or injection. An essential feature of such local carcinogenic action was the long delay in the appearance of the tumour, the "latent period" being characterized by nothing more than a persistent hyperplasia of apparently non-specific nature.

A valuable clue to the mechanism of carcinogenesis was the discovery of a number of substances which, when added to the carcinogenic treatment, profoundly modified the neoplastic response. Some of these (e.g. mustard gas, cantharidin, chloracetone, heptaldehyde) inhibited the carcinogenic effect (i.e. were *anti-carcinogenic*), while others (notably, croton oil, but also turpentine, wound healing, etc.) augmented carcinogenesis when the carcinogen was allowed to act under sub-optimal conditions (i.e. were *cocarcinogenic*).

By testing the cocarcinogenic effect of croton oil during different phases of the latent period, it was found that the augmentation was elicited during the late stages, or after discontinuing the carcinogenic treatment, but not when preceding such treatment. In other words, croton oil could complete the carcinogenic process, but could not initiate it. From these, and related experiments, it became apparent that the previously accepted concept of carcinogenesis as a single, long-continued process, was no longer tenable. Instead, carcinogenesis had to be considered as comprising at least two independent processes: (1) an initiating action, specific in character, involving, pre-

sumably, a sudden transformation of normal cells into "latent tumour cells", and (2) a promoting action, less specific in character, functioning very slowly throughout the latent period, and responsible for the actual evolution of the visible tumour.

Similar conclusions were reached from independent studies of the regression of skin tumours, which could be made to reappear *at the identical sites* by subsequent treatment with a carcinogen or with non-carcinogenic forms of stimulation.

The nature of the initiating and promoting actions are not yet clearly understood. The idea that the initiating action may be mutational in character, though highly attractive and consistent with the irreversibility of the phenomenon and the rapidity with which the change is brought about, is not fully supported by other evidence. Similarly, the idea that the promoting action arises from active cellular proliferation of the previously initiated latent tumour cells, is rendered unlikely by the evidence that not all substances capable of stimulating cellular proliferation act as promoting agents. An interesting observation is the fact that caloric restriction of the diet can profoundly inhibit the promoting action, but not the initiating action.

Other aspects of promoting action were discussed, including the theory that the effect might be dependent on derangements of the mitotic cycle, and an alternative concept, not supported by more recent work, that the promoting effect resided in the subcutaneous tissues (ischaemia, fibrosis, etc.) rather than in any direct influence on the latent tumour cells themselves.

New concepts of "biological autonomy", at a later stage in carcinogenesis, were also discussed.

Prof. Ernst D. Bergmann, *of the Scientific Department, Israeli Ministry of Defence, Tel Aviv,* spoke on "The Role of C_1 Units in Biosynthesis".

Carbon dioxide is not only the source of organic matter in plants and photo-synthethic bacteria; all living cells require this C_1 compound for growth and metabolism. In most of the reactions by which CO_2 is incorporated into more complicated organic molecules, manganese or magnesium (as in chlorophyll) form part of the enzymes involved.

In later stages of the development of life, less inert C_1 compounds are required. The antibiotic action of chloramphenicol and the mode of action of the sulfa-drugs involve such processes. In the former case, it has been shown that the antibiotic blocks the synthesis of indole from anthranilic acid, which differ from each other by one carbon atom. The normal and only source of this carbon atom is methionine; for the incorporation into anthranilic acid, vitamin B_{12} is required. It could further be shown that the cross-resistance of *E.coli* to chloramphenicol and terramycin is based on the fact that the latter too blocks the indole synthesis from anthranilic acid.

The chemotherapeutic action of the sulfonamides on *E.coli* consists of an interference in the synthesis of the purines which is interrupted at the stage of 4-amino-imidazole-5-carboxamide, i.e. a compound which lacks one carbon atom in comparison with the purine nucleus. Here again, the normal and only source of the missing C_1 is methionine, activated in this case by *p*-amino-benzoic acid. It could be shown that the incorporation of this C_1 unit does not take place on the carboxamide itself; the latter is first converted into its deoxyriboside. In this system, too, vitamin B_{12} is of importance.

It is suggested that similar mechanisms are responsible for the formation of riboflavin from a purine riboside, from which the carbon atom C_8 is removed.

The transfer of the deoxyribose molecule (from cell nucleic acids) to the amino-imidazole-carboxamide is catalysed by an enzyme which has proven not to be specific for the above sugar. The xyloside of thymine which occurs in sponges, is also a substrate for this enzyme which evidently has already been in existence in the period in which the nucleic acids were not specifically derivatives of ribose and deoxyribose.

Prof. Severo Ochoa *from the New York University* lectured on "Enzymatic Synthesis of Citric Acid".

He described the work of his group, which led to the elucidation of this synthesis from acetyl phosphate and oxalacetate. Two steps are involved in this synthesis: 1) The formation of acetyl-coenzyme A from acetyl-phosphate by the enzyme "transacetylase" and 2) the condensation of acetyl-coenzyme A with oxaloacetate to yield citrate and free coenzyme A, catalyzed by the "condensing enzyme". The second enzyme is present in large amounts in yeast and a variery of animal tissues and could be obtained in crystalline form from pig hearts. It was shown that the reaction catalyzed by the condensing enzyme: Acetyl-coenzyme A + oxalacetate $\rightleftharpoons$ citrate + coenzyme A is reversible. This reversibility can be proved by using labelled citrate, which in the presence of coenzyme A and the condensing enzyme forms labelled oxalacetate. The reversal can also be demonstrated by coupling the system with malic dehydrogenase and $DPN_{red.}$ giving an overall reaction: Citrate + CoA + $DPN_{red.}$ $\rightleftharpoons$ acetyl—CoA + malate + $DPN_{ox.}$

This reaction can be followed spectrophotometrically, at $\lambda = 340$ m. The rate and extent of this reaction depends on the amount of CoA added and can be carried further by the addition of phosphate and transacetylase, which react with the acetyl-coenzyme A to give acetyl-phosphate and free coenzyme A. The latter can now react with additional amounts of citric acid. The acetyl-CoA formed by the reaction catalyzed by the

condensing enzyme can also act as acetyl donor to sulfanilamide and to choline. A sample of acetyl-CoA obtained by Lynnen from yeast acting on acetate can be shown to donate acetate to the choline acetylation system, and in other systems involving "active" acetate.

Prof. David Rittenberg, *from Columbia University, New York,* lectured on "The Enzymatic Catalysis of some Transformation Reactions".

Experiments have been made which are designed to give information on the transformations occurring in the substrates during enzyme action. Examples were shown of enzymes acting on substrates or on part of the specific substrate under conditions in which no usual reaction can be observed. However, the activation process can be demonstrated by changes in isotope composition. For instance, incubation of chymotrypsin with N-aryl aromatic amino acids did not bring about the splitting of a peptide bond by the enzyme. It could, however, be shown that the carboxyl group of the substrate was activated and the oxygen exchanged with that in the medium, containing $H_2^{18}O$. No such exchange was found in the absence of enzyme. Similarly, L-leucine, containing nitrogen-15 and excess deuterium on the α carbon atom, when incubated with minced pig heart lost most of its deuterium, but none of its N-15. No exchange was found with D-leucine. In the presence of keto-glutaric acid, under the same conditions, N-15 is also exchanged. Similar results were obtained when an esterase was incubated with acetic acid, instead of an ester.

These results were interpreted, to show that an activated structure is formed prior to the complete chemical reaction. A hypothesis was produced to explain the mechanism of this activation reaction.

Dr. Benyamin Shapiro, *of the Hebrew University-Hadassah Medical School,* lectured on "Active Absorption of Sugar".

He discussed the evidence on which the phosphorylation theory of sugar absorption in the kidney and intestine is based and came to the conclusion that this evidence is inconclusive.

Experiments with glucose absorption by an intestinal loop *in vitro* led to the assumption that glucose was not transferred across the intestinal wall in its original form, but in the form of a metabolite. This assumption was verified by the findings that during glucose absorption from the intestine, the glucose concentration in the vena porta was not higher than that in the arterial system. Furthermore, with the intestine perfused *in situ* the glucose disappearing from the lumen could not be found in the perfusion fluid. When radioactive glucose was introduced into the lumen, a radioactive substance can be found in the portal blood. This substance is not reducing and is not fermented by baker's yeast. It makes up 30—60% of the glucose disappearing.

It seems indicated that active transfer of glucose occurs by the formation of a metabolite in the intestine. This metabolite is transported by the portal blood and is changed back into glucose by some other organ, presumably the liver.

Prof. Heinrich Waelsch, *of Columbia University and the New York State Psychiatric Institute,* lectured on "Recent Concepts of the Mechanism of Peptide Synthesis".

He dealt with developments in a field of central interest, that of the synthesis of proteins. In this connection this work is concerned with the manner in which amino acids are shifted by enzymes from one peptide to another and thus catalyze and pattern the growth of the peptide chain. He suggests that such transfer reactions, which are based in part of glutamyl peptides play a key role in protein synthesis.

Dr. Hanan Meyer, *of the Department of Biological and Colloidal Chemistry, the Hebrew University, Jerusalem,* lectured on "The Peptide-splitting Enzymes Found in Brewer's Yeast", a study carried out by A. Fodor, H. Meyer and R. Meshulam of the same department.

Di- and tripeptidase-splitting enzymes were found in yeast autolysates by Fodor and his collaborators as early as in 1927[1]. Later work by Grassmann and Dyckerhoff confirmed their observations[2]. Smith[3] and Johnson[4] later employed sedimentation and electrophoretic methods for the further purification of the crude enzyme extracts. Johnson obtained an electrophoretically homogenous preparation which, however, could not be crystallized. As a reault of the work mentioned above, it can be concluded that the tri- and dipeptidases occurring in yeast macerates are enzymes having roughly the same specificity as the leucine-aminopeptidase and aminotripeptidases found in animal tissues but differ from them by the fact that they are not activated by Mg++ and Mn++ ions. This paper deals with work undertaken by the authors in order to elucidate the somewhat unusual properties which yeast peptidases show on extraction and purification especially with regard to their specificity.

Experimental methods

a) Preparation of enzyme exracts from yeast cells:

Dried brewers' yeast from France (Givaudon), from Czechoslovakia (Olmutz) and for the more recent work, locally produced fresh brewers' yeast were all found suitable.* Autolysis, without which no active extracts could be obtained, was carried out by incubating the yeast cells with five to ten times their weight of distilled water at 37°C for three hours. The fresh yeast could not be treated

* We wish to express our appreciation to Dr. Heimann, chief chemist of the Nesher Breweries at Rishon le Zion (Israel) for a generous supply of yeast.

successfully in this manner but had to be acetone-dried before it was autolyzed. This was done by the Umbreit[8] method. It should be noted that fresh living yeast cells could split casein, peptone and di- and tripeptides, but did not excrete any enzymes into their surroundings, all the activity apparently being concentrated in the cell surface.

Fractionation of the crude enzyme preparation was accomplished in the following manner: The crude extract was brought with N/10 HCl to a pH of approximately 5.5, whereby an active precipitate was obtained (precipitate 1). Since the supernatant was still active, the pH was brought down to 4. A further precipitate resulted which had the same enzymatic properties as that obtained at the higher pH (precipitate 2), but this time no activity was left in the supernatant. Since acid precipitation alone did not bring about a separation of the different enzymes, the following adsorption and elution procedure was worked out:

The crude enzyme extracts were treated with kaolin which adsorbed all the active substances. After thoroughly washing the adsorbates, the enzymes could be eluted with 2% glycine solutions. The same method was used to fractionate the enzymes present in the acid precipitates 1 and 2. Here, the precipitates were washed acid-free and redispersed in water. The enzymes present in these dispersions could be adsorbed on kaolin and eluted with glycine solutions. Glycine was the only substance that could be used as an eluant; all other amino acids and the commonly employed acid or basic buffer solutions were ineffective.

The enzymatic properties of the different preparations are summarized in the following table.

Detailed experimental procedures are given elsewhere[5,9].

It appears from the experimental results shown in Table I that the proteinase activity is lost both upon precipitating with acid and on adsorption. Peptone-splitting activity survives acid precipitation but is lost upon adsorption on kaolin. Tripeptide-splitting activity is present in Eluate B, but not in Eluate A, which in addition has lost the ability to split glycylpeptides.

The following properties of the different eluates have also been observed: Prolonged dialysis of the eluates against distilled water, until the disappearance of the Ninhydrin reaction (indicating the absence of glycine) led to the almost total loss of the enzymatic activity, which could be partially restored by the addition of Cl⁻, total reactivation took place upon the addition of ultrafiltrates of crude yeast macerates but not when the added ultrafiltrates were previously treated with kaolin. The addition of glycine to the dialyzed Eluate B led to a further activation of LGG-splitting activity (30%).

The protein content of all the eluates was exceedingly low, so low that no qualitative protein reactions (Esbach and Spiegel) could be obtained. Activity was not decreased on treating the eluates or the crude enzyme extracts with 5 times their volume of dialyzed ferric hydroxide sol which precipitated most proteins.

As already mentioned, tripeptidase activity was enhanced by Cl⁻ ions. Activation could also be obtained by adding Zn⁺⁺ ions (M/1000).

A 40% inhibition of dipeptidase activity was observed on adding M/1000 ferricyanide; tri-

TABLE I

Fractionation of the peptide-splitting enzymes present in yeast macerates

Autolyzed yeast macerate (attacks casein, peptones, leucylglycylglycine (LGG), leucylglycine (LG), glycylleucine (GL)

Precipitate at pH 5.5 (Precipitate 1)	Precipitated at pH 4 (Precipitate 2)	Adsorption on kaolin
		Elution with glycine solution
(splits peptone, LGG, LG, GL, but not casein)		(Eluate A)
Redispersion in water, adsorption on kaolin and elution with glycine solution		Splits LGG and LG, but no GL, peptone or protein
Splits LGG and GGL, but no dipeptide, peptone or protein (Eluate B)	No activity can be eluted with glycine soln.	

peptidase activity was not inhibited by this reagent. Addition of Pb++ ions (M/1000), however, caused almost total abolition of the tripeptidase activity, leaving the dipeptidase activity intact.

Discussion

The experimental evidence seems to indicate that at least a considerable part of the proteolytic and peptide-splitting activity of the yeast cell is localized in its surface. Furthermore, proteolytic activity of yeast cells demands the existence of normal respiration since dead cells are unable to split protein or peptides. The enzymes in or on the cell surface are inactivated or denatured on killing the cells and can be reactivated only by an autolysis of several hours duration. They are brought into solution as a phosphoprotein complex which was first described by Fodor[1] and later by Johnson. It is, however, uncertain whether this protein is to be regarded as a more or less pure or even a single enzyme, since it was found by Johnson that even partially deactivated preparations still were electrophoretically homogenous. The adsorption and elution procedure developed by Fodor leaves little doubt that the phosphorprotein cannot be the enzyme itself because no such protein and especially no phosphorus could be found in the active eluates. One must therefore assume that the phosphoprotein is the carrier of enzymatically active substance which can be separated from it by adsorption on kaolin and subsequent elution, the carrier protein remaining absorbed on the kaolin. The proteinase activity present in the autolysates before adsorption seems, however, to be bound to their protein since no protein-cleaving activity could be found in any of the eluates.

It has already been pointed out that the eluates obtained by us do not always have the same specificity characteristics. It is therefore assumed that the substance eluted with glycine is somewhat similar to a coenzyme which is in itself inactive, but gains activity according to different apoenzymes present. The theory is proposed therefore that there exists orginally in the yeast cell wall a large protein complex with a number of active centers on which the coenzyme(s) are adsorbed. This complex is, in the living cell, capable of accomplishing all the protein and peptide hydrolyses needed by the cell. Killing the cells destroys the "dynamic state" of this protein, and the stable compound so produced is enzymatically inactive.

On autolysis, the coenzyme is liberated, and at the same time suitable carrier substances are produced with which the coenzyme can produce the different specificities observed in the autolysates. Whether these enzymes are all part of a single labile complex, the main part of which consists of the phosphoprotein already mentioned, or separate entities, remains an open question. The assumption of a large complex, however,

seems made more probable by the fact that partial denaturation (precipitation) at pH 4 affctes all the enzyme activity, since all activity originally contained in the protein fraction precipitated at pH 4 can be adsorbed on kaolin, but cannot be eluted. More careful precipitation (at pH 5.5) or no acid treatment at all leaves the protein complex in a more labile state and allows it to be split into its different components by the influence of the eluant, according to their respective affinities for it.

An attempt should be made here to explain the rather surprising specificity of glycine as an eluant. In view of the fact that all the aminopeptidases yet investigated act on substrates containing one or more glycine residues, it seems to be not unreasonable to suppose that the glycine when used as an eluant combines with such places on the enzyme molecule surface originally acting as active centers and suited for combination with glycine. By combination with the eluant, the bond enzyme-adsorbent is sufficiently weakened to allow the enzyme(s) to pass into solution. The eluted complex enzyme-glycine is not very stable since it does not seriously interfere with the normal enzymatic activity developed when a suitable substrate is added, whereby the glycine is displaced. This can be suitably interpreted in terms of Bergmann's "polyaffinity theory" which postulates three points of interaction of a peptide with its enzyme, one of which should be the side chain of an amino acid. The side chain binding activity of the different amino acids was calculated by Smith and Lumry who found that this constant was the lowest of all for glycine (400 cal/mole) and at least ten times lower than that of alanine, the next lowest one. It is therefore not surprising that successful elution of the enzymes could not be achieved by any other amino acid besides glycine. All other amino acids apparently form stable complexes with the enzyme, thus inactivating it.

Conclusions

Concluding the discussion on the experimental evidence so far gathered, the authors feel justified to express the opinion, that the rather variegated specificity properties of the proteinases and peptidases in yeast are not due to the coexistence of a single (or at most very few) coenzyme active substances, which gain their specificity owing to their combination with suitable apoenzymes. This problem is parallel to the case of the iron-containing enzymes, carrying the heme system as their prostethic group, where the specificity is also largely due to the characteristic properties of the respective protein carriers.

REFERENCES

1. FODOR, A. and SCHOENFELD, R., 1927, *Hoppe-Seyl. Z.*, 170, 231.
2. GRASSMANN, W. and DYCKERHOFF, A., 1928, *ibid*, 179, 41.
3. SMITH, E. L., 1952, *Advances in Enzymology*, 12, 191 ff., Interscience, New York.

4. JOHNSON, M. J. and BERGER, I., 1942, *ibid*, **2**, 69 ff.
5. FODOR, A. and FRANKENTHAL, L., 1930, *Biochem. Z.*, **229**, 16.
6. FODOR, A. and FRANKENTHAL, L., 1931, *ibid.*, **232**, 283.
7. UTTER, M. F. and WERKMAN, C. H., 1942, *Biochem. J.*, **36**, 485.
8. UMBREIT, W. W., 1949, in UMBREIT, *Manometric techniques*, Burgess Publishing Co., p. 128.
9. MESHULAM, R., 1952, *M. Sc. Thesis*, The Hebrew University, Jerusalem.
10. JOHNSON, M. J., 1941, *Jour. biol. Chem.*, **137**, 575.
11. BERGMANN, M. and FRUTON, I. S., 1937, *Jour. biol. Chem.*, **117**, 189.
12. SMITH, E. L. and LUMRY, R., 1950, *Cold Spring Harbour Symp. Quant. Biol.*, **14**, 168.

Dr. Kurt G. Stern, *of the Polytechnic Institute of Brooklyn*, lectured on "Plasma Proteins and Plasma Substitutes".

Following a brief discussion of the nature and function of the normal plasma proteins, some recent studies dealing with physical-chemical properties of protein components, present in normal and pathological blood plasma, were reported. It was shown that the protein distribution in normal human sera is a function of the age of the subjects. The recent work by Gofman, dealing with macromolecular lipoprotein components associated with the incidence of atherosclerosis, was reviewed. Joint studies with M. Reiner on the electrophoretic analysis of the sera of multiple myeloma patients have shown the statistical preponderance of the so-called gamma plasmocytoma pattern where the anomalous globulin component migrates with the mobility of gamma globulin without, however, being identical with normal gamma globulin. Bence-Jones proteinuria was found to be present in less than fifty per cent of the ninety cases studied.

The plasma substitute or plasma extender under study in the speaker's laboratory at present is Dextran, a polysaccharide of bacterial origin which has been degraded to a suitable, average particle size by chemical and physical methods. The latter include treatment with sonic vibrations and bombardment with high-intensity electron beams of short duration, generated by the Capacitron of Brasch. The native, high-polymer dextran and the partially degraded preparations obtained from it have been studied in the analytical ultracentrifuge, in the diffusion apparatus, as well as by light-scattering, viscosity, and flow birefringence techniques. Electrophoretic experiments on the blood serum of patients, infused with dextran of clinical type, indicate that the material does not enter into complex formation with the protein components present in blood serum. The enzymatic synthesis of dextran by cell-free, purified enzyme preparations from sucrose as substrate is under active investigation.

Prof. Arie Olitzki, *of the Hebrew University-Hadassah Medical School*, lectured on "The Use of Streptomycin-resistant and Dependent Mutants of *Brucellae* in Immunization Experiments".

Streptomycin-resistant mutants of *Brucella abortus*, *B.melitensis* and *B.suis* were found to be as virulent as their parent strains. The virulence determined on mice with the aid of the mucin technique was correlated with the appearance of the colonies. Four different mutants were described: 1. Streptomycin non-resistant, smooth, virulent, 2. Streptomycin non-resistant, non-smooth, avirulent, 3. Streptomycin-resistant, smooth, virulent, 4. Streptomycin-resistant, non-smooth, avirulent.

The M.P.N. (most probable number) method was employed in order to determine the rate of appearance of streptomycin-resistant mutants. The results were already described elsewhere[1].

Streptomycin-resistant mutants were employed in studies on the immunizing effect of living *Brucellae*. When the immunizing strain was non-resistant and the challenge strain streptomycin-resistant or *vice versa*. then it was possible to determine quantitatively whether the immunizing strain was still present in the organs and where the challenge strain was able to get a foothold after the primary infection had taken place. Different degrees of immunity were observed.

A streptomycin-dependant strain did not multiply in mice, but the micro-organisms could still be isolated four weeks after their inoculation from the spleen. The strain did not produce pathological changes but produced some degree of immunity when administered in doses of 10^8 to 10^9 micro-organisms. No clinical symptoms were observed on a volunteer who received 2×10^7 living streptomycin-dependant *Brucellae*.

REFERENCE

1. OLITZKI, A. L., 1952, *J. Gen. Microbiol.*, **6**, 166.

Dr. Nathan Grossowicz, *of the Hebrew University-Hadassah Medical School*, lectured on "A Microbiological Approach to the Nutritional Evaluation of Proteins"[1].

The nutritive value of a protein is largely determined by the concentration and proportion of the various essential amino acids liberated from it by the action of proteolytic enzymes. However, the mere comparison of different proteins on the basis of their amino acid composition, obtained by chemical hydrolysis, is not an adequate criterion for estimating biological activity. If the chemical hydrolysis is carried out properly, the protein is totally decomposed to its different amino acids. The actual liberation of the amino acids which is determined by the digestibility of the protein, is not considered at all. It is the digestibility, however, which determines the nutritional value of the protein.

A microbiological method to assess the nutritional value of proteins is described. The method implies the digestion of the proteins with *pancreatin* and subsequent testing of the growth-promoting activity of the hydrolysate for *Streptococcus faecalis*, in a medium devoid of the 10 *essential* amino acids. The nutritive value of the protein

digest is determined in a *single* assay by the particular amino acid liberated in a limiting concentration.

The results obtained with casein, egg albumin, gelatin, gluten and zein are presented and their agreement with the biological values obtained by the rat method is indicated (Table I).

TABLE I

Comparative growth of *Streptococcus faecalis* in pancreatin hydrolysed proteins

	Amino N γ/ml	Klett reading filter No.660	Relative activity casein =100 %	Rat growth test[2] casein =100 %
Egg albumin	17.5	100*	105	119
Casein	17.5	95	100	100
Gelatin	60.0	90	29	—
Gluten	87.5	70	15	16
Zein	175.0	20	2	—

* The tests were carried out in the Henderson-Snell medium[3] from which the 10 essential amino acids were omitted and one of the protein hydrolysates incorporated. The growth of the test organism in the complete medium[3] gives a turbidity reading of 220—250.

The growth promoting activity (biological value ?) of casein and egg albumin is high and about equal; gelatin and gluten in comparison are much less active, 29 and 15 per cent respectively, and zein still less (2 per cent only).

At a limited growth level, casein is deficient in lysine, arginine, isoleucine and threonine. Egg albumin under comparable conditions is limited in lysine, arginine, histidine, threonine, isoleucine, leucine and valine. Gelatin is low in tryptophane, isoleucine, leucine, methionine and threonine.

Data were presented to show that the method may be used for the study of imbalances of amino acids.

This work was carried out in collaboration with Mr. Sam Halevy.

REFERENCES

1. GROSSOWICZ, N. and HALEVY, S., 1952, *Bull. Res. Counc. of Israel*, 2, (in press).
2. Bureau of Biological Research, Rutgers University, New Brunswick, N. J., 1950, *Report on cooperative determinations of the amino acid content, and of the nutritive value of six selected protein food sources*, p. 50, 53.
3 HENDERSON, L. M. and SNELL, E. E., 1948, *J. biol. Chem.* 172, 15.

Prof. Manfred Aschner, *of the Hebrew-University-Hadassah Medical School, Jerusalem*, lectured on "Intracellular Growth of Micro-organisms in Insects".

Problems connected with intracellular growth of micro-organisms were discussed, and as a particular case, the symbiotic bacteria of insects were described in detail.

The intracellular symbionts, in contrast to parasites, do not attack the cytoplasm of the cell in which they live but rather utilize the waste products of the metabolism of the cell in such a way that a well-balanced equilibrium between host and guest is possible. As an example, the symbiont of the scale insect, *Pseudococcus citri* was cited,

which according to Fink[1], is able to live in a very simple media in which urea is the sole source of carbon and nitrogen.

Reproduction of the symbiont is regulated, as has been observed in so many instances by Buchner[2] and his school, by the needs of the host organism. For instance, there is an increased rate of multiplication of the symbiont at the time the symbionts are supplied to the developing eggs of the host. The above-mentioned dependency of the intracellular symbiont on the activity of the host cell is probably the mechanism for this regulated behaviour.

By experimentally freeing lice[3] and certain beetles[4,5] of their symbionts, it could be shown that these insects would survive without their symbionts only if vitamins of the B group and certain amino acids and sterols were supplied in the diet. It may be concluded from these facts that one of the functions of the intracellular organisms is to supply these substances to the host organism.

Obligatory intracellular life obviously does not reduce those synthesizing abilities of the intracellular inhabitant which are usually affected by a parasitic mode of life. This is another proof that there is a basic difference between intracellular symbionts and parasites.

REFERENCES

1. FINCK, R., 1952, *Z. Morph. oekol und Tiere*, 47, 78.
2. BUCHNER, P., 1952, *Tier und Pflanze in Symbiose*, Basel, 3rd ed. (in press).
3. ASCHNER, M. and RIESS, E., 1933, *Z. Morph, oekol und Tiere*, 26, 529.
4. KOCH, A., 1933, *Biol. Zbl.*, 53, 199.
5. FRAENKEL, Y. and BLEWETT, M., 1943, *Biochem. J.*, 37, 642.

Prof. Judah H. Quastel, *of the McGill University, Montreal*, lectured on the "Biochemical Aspects of Narcosis".

The action of narcotics on enzyme systems and respiratory processes was discussed. In the past, the relationship between narcosis and oxidative events was doubted, since it was found that quantities of narcotics required to induce narcosis are usually much smaller than those required for inhibition of enzyme actions. However, it might be possible that the drug effect was localized, so that no considerable change in respiration of the entire central nervous system can be found. With the brain slice technique, using only brain cortex it could be shown that definite inhibiton of respiration takes place in presence of narcotics at concentrations that produce narcosis in animals. Narcotics do not inhibit all oxidative processes to the same extent. Oxidations of glucose, pyruvate and lactate are most affected, while those of succinate and *p*-phenylenediamine are undisturbed. Low concentrations of narcotics, which are highly inhibitory aerobically, have no effect anaerobically. It can be concluded that narcotics do not affect the primary activation of the substrates by dehydrogenases, nor do they inhibit the terminal oxygen activation (cytoch-

rome oxidase). The narcotics have an affinity to a special component playing an important part in the chain of reactions constituting the complete aerobic respiratory process of the nerve cell. Concommitant with the depression of respiration, acetylcholine synthesis is depressed. However, no effect was observed on the anaerobic synthesis of acetylcholine in brain extracts enriched with ATP. No phosphorylation induced by ATP could be shown to be affected by the narcotics. The most obvious explanation of these results is that narcotics inhibit those links in the respiratory chain responsible for oxidative synthesis of ATP. All those activities of the nerve cell which are dependant on high energy phosphate bonds will thus be affected.

An entirely different mechanism is involved in the action of morphine. Morphine competes with acetylcholine for receptor groups, essential for the normal functioning of the nerve.

Prof. David Nachmansohn, *of Columbia University*, in his lecture "The Metabolism and Function of the Nerve Cell", described his widely-known contributions to an understanding of the chemical and physical basis of nerve conduction. He showed how a special enzyme (acetylcholine esterase), which is present in enormous concentration in the nerve tissue, plays a central role in the mechanism responsible for the generation and transmission of the electrical impulses. One of the experiments described by Prof. Nachmansohn was of particular interest in that it settled a longstanding controversy in this field. Drugs such as acetylchlorine and curare have been succeefully injected into the nerve axon and thereby demonstrated that these substances are able to act not only at the nerve endings but in the nerve upon itself.

Prof. Felix Bergmann *of the Department of Pharmacology of the Hebrew University-Hadassah Medical School* lectured on "The Nerve Membrane as an Ion Exchanger".

The inhibitory effect of quaternary ammonium salts is a function of molecular size of the cation. The relationship between these competitive inhibitors and the enzyme thus resembles their behaviour towards cation exchange resins. The inhibition by all quaternary ammonium ions decreases towards zero, when the pH changes from 7 to 5, indicating that the anionic site of the active surface is inactivated through combination with protons.

The enzyme behaves therefore like a cation exchanger, whose affinities are a function of pH. The selective permeability of the nerve membrane for different cations can thus be deduced from the ion exchange properties of the enzyme which forms part of the functional nerve membrane. During conduction this specific permeability breaks down. Such a change can be ascribed to a change of pH, which involves a change in the affinity of the anionic sites and a change in the network structure of the protein, (since this is dependent on hydrogen bridging). It is thus concluded that the action current produces a shift of hydrogen ions in the nerve in such a way, that the pH of the membrane decreases and the selective permeability breaks down.

In order to restitute the normal potential, all conditions must be returned to the original status. This will result automatically, if the pH can be returned to its resting value. For this purpose a reaction must take place which is very fast, but not instantaneous (e.g., inorganic buffers would be unsuitable). A suitable substrate can be found in the system acetylcholine—choline esterase, which can act as an "enzymatic buffer", since the equilibrium of the reaction

$$\text{choline} + \text{acetate} \rightleftharpoons \text{acetylcholine} + \text{water}$$
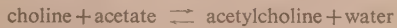
has been shown to be a function of the pH.

Prof. Israel S. Wechsler, *of New York*, attempted to describe in his paper, "Clinical Hypothalamic Syndromes Anatomico-Physiological Considerations", one clinical hypothalamic syndrome which is characterized by a certain type of coma previously alluded to as coma vigil. In this state of coma the individual may keep his eyes open, the eyeballs may wander, and he may give the impression that he is awake and in contact with his environment though he is not. Generally there are no focal signs and there is no evidence pointing to other regional involvement of the brain. The patient may be extremely restless and give evidence of sexual excitement or general psychotic behaviour. In addition, there may be and often are one or more signs or symptoms reflecting disturbance of function of the diencephalon. Among these are fever, often very high, not the result of infection. There may be hypothermia. If fever is present it is unaccompanied by its usual manifestations. There is want of parallelism between fever and pulse rate, the latter often being low when the former is high; that is, there is dissociation between them. The fever may respond to barbiturates when it does not to antipyretics. There may be excessive, indeed extremely profuse, perspiration in the absence of fever. The blood pressure may drop to normal or below in known hypertensives or fluctuate in either direction in individuals who were known to have normal blood pressure. There may be polyuria, transient glycosuria and hyperglycemia in non-diabetics. Occasionally one observes minor twitchings, at times a major convulsion.

The condition may occur on the basis of vascular disease or in infections, often an encephalitic process. It may be the result of trauma to the brain, especially of the base. It may be one aspect of other pathologic processes, more particularly tumours, which impinge on the interbrain region. The syndrome may occur in the course of tumor

of the third ventricle, craniopharyngioma, or other neoplasms at the base in the middle fossa. It may occur in the course of diabetic or other encephalopathy. The condition is always grave and frequently ends fatally. It may end in recovery, in which case, if the pathologic process was limited to the hypothalamus, there need be no residue. The coma may last days, weeks or months and end either in death or in recovery.

Several minor diencephalic syndromes have been alluded to, namely diabetes insipidus, behaviour disorders, variations in states of consciousness, fever of unknown origin, and petit mal seizures. The latter may be characterized by extreme pallor, drop in blood pressure and rise in pulse. This type of petit mal may be favourably affected by ephedrine or dexedrine and not by sedative anticonvulsants; sometimes a combination of the two may be effective when neither alone is.

The paper includes a brief summary of the anatomy of the hypothalamus, of its numerous afferent and efferent pathways and their connections with other parts of the brain, and of the blood supply. Extensive consideration is given to the neurophysiology of the hypothalamus including discussion of the metabolic functions of the interbrain structures and their relationship to states of consciousness and to mental and emotional disturbances.

This being a clinical study evidence of pathology was purposely left out, and not because it is unimportant. Attempt has been made to correlate clinical observations with regional anatomy and experimental physiology and pathology. Symptoms were interpreted in the light of what is known so far of the normal function of the hypothalamus and of the manifestations resulting from expermentally induced lesions or from stimulation experiments. Further studies may throw light on the correctness of the observations and validity of the conclusions.

Dr. Alexander Geiger, *of the Hebrew University-Hadassah Medical School*, in his paper, "The Rates of Exchange and Sources of energy in the Living Brain", presented a summary of recent developments with the use of the Geiger-Magnes brain preparation as a tool in the study of brain metabolism. He was concerned particularly with the role of respiration in the maintence of brain function, and showed that wide latitude of variation in oxygen supply and respiration rate is tolerated by the brain without prejudice to function; and that, in the absence of glucose, the brain may actually shift to some other fuel as the source of its required energy. In conclusion, Dr. Geiger made the interesting point that a substance secreted by the liver plays an important role in the control of glucose uptake by the brain, thus stressing the possibility that organs outside the brain have a part in maintaining brain function and showing that disturbances at these sites may be involved in neurological disturbances.

*

At the close of the sessions, Professor Fieser and Wechsler delivered farewell addresses to which the Rector, Prof. Moshe Schwabe, responded on behalf of the Hebrew University.

SYMPOSIUM ON PHOTOSYNTHESIS

On the 18th November the Jerusalem Branch of the Israel Chemical Association held a symposium on photosynthesis.

In his introduction, the chairman of this session, Dr. I. Leibovitz, stressed the importance of photosynthesis. which exceeded by far the output of the largest chemical industry, that of steel production, even without taking into account photosynthesis in the sea. He then briefly described the history of the development of knowledge about photosynthesis from its discovery to the present day.

Dr. G. Stein began by pointing out the tremendous amounts of energy which sunlight gives per unit area. He went on to discuss the utilization of this energy through a photochemical reaction by which sunlight is absorbed by chlorophyll and then utilized in the decomposition of water into hydrogen (which combines with a suitable acceptor) and oxygen which is liberated. The possible reaction mechanisms, involving electron transfer processes, were described. Discussing the energetics of this reaction he showed that what is unusual is not the photochemical decomposition, but its unidirectional nature, the reverse re-action apparently not taking place. He showed that in other photochemical reactions the back-reactions play an important part and suggested that the irreversibility of the photochemical decomposition of water in photosynthesis may be due to the spatial separation of the different components of the system within the living cell.

Dr. A. M. Mayer discussed the photochemical decomposition of water by isolated chloroplasts, the Hill reaction. He recalled the work proving that the oxygen liberated originates in the water. In continuation he discussed rhe analogy between the Hill reaction and the liberation of oxygen in the complete plant cell. He stressed that in view of the similar effects of specific inhibitors, the similar kinetics and reaction products and in view of the work of Ochoa and Vishniac showing that a purely biological system can in the presence of chloroplasts bring about the decomposition of water in the presence of light, the Hill reaction and the photochemical reaction of photosynthesis may be regarded as identical. The lecture was concluded by a short survey of the effects of CO_2 concentration, light and temperature on the rate of photosynthesis.

Dr. B. Shapiro discussed the problems of carbon dioxide fixation. He began by pointing out that following the work of Wood and Werkman it has been shown that tissues from all organisms examined could fix CO_2. He showed the experimental difficulties of proving CO_2 fixation by systems containing pyruvic acid and $TPN.H_2$ due to the unfavourable equilibrium constant of the reaction. Recalling the work of Ochoa he showed how this can be overcome by adding a system which continuously removed the TPN formed. He went on to show that mechanisms of this or a similar nature could lead to CO_2 fixation by compounds of the Krebs cycle from C_2 to C_5, and discussed the significance of these reactions with regard to photosynthesis. Similar reactions might exist in photosynthesis, the only difference being that the energy would be derived from the photochemical reaction. In continuation he reviewed the work of Benson and Calvin who showed that the earliest product of photosynthesis which could be isolated was phospho glyceric acid, and pointed out that this evidence did not exclude the possibility that CO_2 fixation in the plant followed the paths suggested.

A lively discussion followed with regard to the implications of these suggestions on the process of photosynthesis.

ISRAEL CHEMICAL ASSOCIATION

The 14th Annual Convention of the Israel Chemical Association took place on December 15—17th, 1952, in Tel-Aviv. The opening session was devoted to the memory of Dr. Ch. Weizmann, who has been Honorary President of the Association since its existence. Dr. Ernst D. Bergmann described in this Presidential Address Dr. Weizmann's scientific work and subsequently discussed the "Similarities and Differences between the Synthetic Methods of the Laboratory and the Living Cell".

The first half of the Convention was devoted to a Symposium on "Sugars". In its framework Dr. J. Leibowitz discussed "Rare Sugars", Dr. S. Hestrin the "Enzymatic Synthesis and Breakdown of Starch", Prof. A. Katchalsky the "Physical Chemistry of Polyuronic Acids". Mr. N. Sharon spoke about the "Reaction of Aldoses with Basic Polyamino Acids and Proteins", Prof. M. Aschner about "Raw Materials for Technical Fermentations", Dr. D. Aminoff about "Mucoids and their Biological Functions" and Mr. U. Z. Littauer on the "Metabolism of Pentoses".

In the second half of the Convention, 47 original papers were read, distributed between three sections (inorganic and physical chemistry, chemistry, biochemistry). Each section held three sessions. The lectures, which were followed by lively discussions, gave an instructive picture of the work which is being carried out in the various research institutions of the country. The abstracts of the original papers appear in this issue of the Bulletin of the Research Council of Israel.

A special session of the Convention dealt with organizational problems. The formation of a Section of Analytical Chemistry was announced. The President and the Executive Committee of the Association were re-elected.

SYMPOSIUM ON THE PROTECTION OF NATURE AND ANTIQUITIES

A symposium organized by the Research Council of Israel in cooperation with the Botanical and Zoological Societies took place in Tel Aviv on December 15, 1952. It was devoted to various aspects of nature protection and preservation of places of historical and archaeological interest.

The symposium was opened by Mr. N. Landau of the Research Council, who stressed the scientific importance of nature protection and outlined the educational, legislative and executive measures required.

Prof. B. Mazar, Rector of the Hebrew University called for an ambitious educational campaign designed to instill in the population an appreciation of the natural beauty of the country and an understanding of its long and rich history. Legislative action will be of little value unless backed up by a sustained educational effort in schools, in youth movements and in the army. Archaeological and botanical investigations should be carried out before a settlement is built on a new site and valuable treasures are irretrievably lost.

The Minister of Education, Prof. B. Z. Dinour, promised the whole-hearted support of his Ministry for any measures designed to implement the resolutions of the symposium. Mr. Y. Weitz of the Jewish National Fund proposed a dynamic approach to the problem of nature protection. He suggested foreign introduction and extensive afforestation which will even alter the character of the landscape. Mr. S. Yevin, Director of the Department of Antiquities stated that some 2000 sites of archaeological importance are known in Israel and there are probably many more still to be discovered. It is most distressing that priceless treasures are being destroyed as a result of ignorance and neglect.

Dr. W. C. Lowdermilk of F.A.O. maintained that soil conservation was the foundation of nature protection. Many great civilizations ceased to exist in consequence of soil erosion. In arid countries like Israel, soil conservation and cont-

rol of rainwater are essential for the maintenance of agricultural production.

Mr. O. Gil of the Ministry Agriculture gave details of the extent of soil erosion. A soil survey of some 10.5 million dunams is being completed and the Ministry is engaged on projects of soil conservation in various parts of the country. It is interesting to note that in the Sdeh Boker area in the Negev where new terraces are being built on top of terraces constructed 2000 years ago, no modern engineer could improve upon the perfection of these ancient structures.

In a lecture supplemented by numerous lantern slides, Prof. M. Zohary of the Hebrew University pointed out the historical importance of Palestine in that three major phyto-geographical regions converge here. Many interesting plant species are threatened with extinction unless adequate measures are adopted to ensure their survival. In the past, woody vegetation was ruthlessly destroyed by the native population so that fine tree specimens could only be found around old Moslem groves. The few remnants of forest still extant are in great danger unless adequate precautions are promptly taken. Dr. Naomi Feinbrun of the

Hebrew University described rare plants, notably the lily found in Galilee and a number of beautiful irises. It will probably be impossible to re-establish these species if they are eradicated.

Dr. M. Mendelsohn of the Tel Aviv Biological Institute listed several wild animals mentioned in the Bible which are now extinct in Israel. He demanded strict enforcement of the hunting laws and the establishment of reserves with a view to replenishing the animal stock. The commercialized destruction of corals in the Elath Bay should be immediately stopped. Mr. A. Zahavi of Jerusalem described the unique animal paradise in the Huleh region and outlined a detailed plan for a reserve totalling about 3000 dunams to be left after the draining of the swamp. Dr. A. Brutzkus of the Planning Authority explained the activities of his department with regard to reserves, national parks and historic sites. The proceedings were wound up by Mr. Y. Hoofien of Tel Aviv.

The resolution adopted called for the setting up of a government authority for the protection of nature with full executive powers and the establishment of a national society of nature lovers.

THE AHARONI PRIZE

On December 18, 1952 the Aharoni Prize was awarded in the Hall of the Manufacturer's Association in Tel Aviv by the Research Council, trustee of a fund donated by the family of the late Ben Zion Aharoni in whose memory the prize is annually awarded for outstanding research on citrus fruit problems.

The panel of judges, appointed by the Research Council consisted of Prof. M. Aschner of the Hebrew Ubiversity, Dr. Koffler of "Yakhin Ltd.", Dr. W. Pilnik of the Central Citrus Fruits Laboratory and Mr. N. Landau of the Research Council of Israel.

Ben Zion Aharoni was one of the pioneers of citriculture in this country. He strove to create strong ties between agriculture and industry and

foresaw the possibility of establishing a ramified food and chemical industry on the basis of citrus by-products. Such developments would naturally strengthen the foundation of our agriculture. Inspired by this vision, the Aharoni Family chose a most fitting way to commemorate his aspirations and achievements, in encouraging original applied research in the field which was so close to his heart.

Last year Mr. I. Zuckerman was chosen as worthy of this prize for his work on the effect of limonene on micro-organisms.

Scientific workers concerned with this field of research are reminded that this year too the prize will be awarded and that papers should be submitted to the Research Council of Israel not later than June 1.

FARKAS MEMORIAL VOLUME

Editors: Adalbert Farkas and Eugene P. Wigner, Publishers: Research Council of Israel, 309 pp.

IL.5.000, $6.00, £2/2/0, November 1952

The collection of papers published by the Research Council of Israel to mark the fourth anniversary of the death of Professor Ladislas Farkas, is a fitting memorial to this remarkable scientist and his work. The volume includes work by his teachers, friends and pupils, people who either worked directly with Farkas ro were influenced by his researches. As Harold Urey remarks at the end of his fascinating article on the

origin of planets: "I sincerely hope that the subject of my paper would have interested him—were he still with us".

It is not possible within the space of a short review to analyse the scientific value of the many and varied articles gathered in this large volume. The articles cover a wide range of research, from theoretical physics to experimental biology, and we can assume that each will be reviewed sep-

arately within its own field. We shall try to indicate how this book illustrates—through the work of Farkas' associates — his own life's work.

The first systematic work of Farkas, including his doctorate thesis, was an investigation into the mechanism of photo-chemical reactions. In a series of papers which appeared between the years 1928 and 1933, he elucidated the inner mechanism of a number of elementary inorganic reactions. Considerable progress was made towards clarifying the influence of light on the participants in the reaction and work was begun on photochemical reactions. This last work was developed several years later by Farkas and his associates in Jerusalem. The photo-chemical work is recalled by the papers of Platzman and Frank on the theory of "the adsorption spectrum of halogen ions in solution"; of Uri on "the reaction of atoms and free radicals and their photo-chemical production"; of Burton on "the mechanism of radio chemical reactions in organic and hydro-organic systems" and the paper by J. Weiss on "the reaction between hydrogen peroxide and ferric salts".

In 1933, Farkas and his brother, Adalbert, found refuge from Hitler in Rideal's laboratory in Cambridge. It was here that they began their classical researches on the ortho-para-hydrogen conversion on para-magnetic substances, work which has kept its importance to this day as providing a powerful indicator for investigating catalytic reactions and for the elucidation of the mechanism of various reactions. About that time Farkas was requested to prepare large quantities of deuterium, and the methods which he developed for this purpose have been used, among others, to provide science with large quantities of heavy water for general mechanical and biochemical research. A number of the articles in the work continue and develop this fruitful line of research with isomers and hydrogen isotopes. The work of Dostrovsky, Gillis and Llewellyn deals with the improvement of the method for "separation of isotopes by fractional distillation", while other works deal with the investigation of fine mechanisms of reaction using methods made possible by techniques worked out by the Farkas brothers. There is the work of Melville and Robb on "fast reactions of atomic hydrogen"; Bauer on "the velocity of very fast reactions"; Eyring, Parlin, Wallenstein and Zwolinski on "some aspects of catalytic hydrogenizations"; Komarewsky, Zimmerschied and Coley on "the reaction mechanism of the catalytic dehydrogenization and condensation of aliphatic alcohols", and the works of Boudart and Taylor on "the hypothetical role of solid adsorbents ... ". In this work, which is devoted to the investigation of adsorption mechanisms by means of hydrogen and deuterium, we see a further branch of the Farkas' scientific work-research of the border region of hetrogeneous systems. Out of this work they developed entirely new possibilities for understanding the influences of solid catalysts on many reactions which were of fundamental importance both in the laboratory and in industry. Along this line of research too we see the works of Pines on "the mechanism of isomerisation of methylcyclopentane" and Nachod on "catalysis by means of ion exchanges". Into this group too falls the work on solid membranes in general including that of Bonhoeffer on "mechanism of the creation of ferrocyanic copper membranes" and that of Mark on "osmometry of polymeric solutions at high temperatures".

The research on the simplest atoms of chemistry—hydrogen and deuterium—provided Farkas with the opportunity to investigate the simplest of those chemical exchange reactions which can be completely described by the equations of quantum mechanics. We are reminded of this work when we find in the present volume theoretical papers such as that of Schwartz on "the importance of elementary reactions for chemical kinetics" as well as fundamental papers on the structure of atomic nuclei such as those of Wigner on "the shell model of atomic nuclei" and of Racah on "nuclear levels and the Casimir operator".

In his last years, Farkas became very interested in applied science in his deep desire to use scientific methods for the development of the country. His applied researches led him into problems of biochemistry and agriculture and here he was able to use isotope techniques. Related to this period of his research we see the work of Yudkin on "enzymatic adaptation as a method for investigating material exchanges"; that of Frankenburg on "fermentation in tobacco leaves and its chemical basis", and that of Gillis, Spitnick and Ephraim Katchalsky on "the theory of the action of hydrolytic enzymes on homogeneous linear polymers".

Alongside this list of research papers, there are also some more general articles such as that of Polanyi on "Dalton's theory" and of Simon on "a simplified formula for melting pressure".

This should suffice to give us an idea of the scope of the book and of the wide field of scientific interest associated with the name of the first Professor of Physical Chemistry in the Hebrew University.

The book is clearly and accurately printed and should augur well for the future publication of serious scientific books in Israel. All those of us who treasure the memory of Ladislas Farkas will welcome this fine work performed by the Research Council of Israel.

Aharon Katchalsky

THE SEVENTH INTERNATIONAL CONGRESS FOR THE HISTORY OF SCIENCE

The Seventh International Congress for the History of Science will be held in Jerusalem on August 4—11, 1953.

In addition to the general sessions on basic problems of humanism, the meetings will be divided into the following sections:

1. The History of Mathematics and Physics, etc.
2. The History of Chemistry and Biology, etc.
3. The History of Technology
4. The History of Medicine
5. General problems of the Method of Science.

All those who wish to participate in the congress should contact the president of the congress, Prof. F. S. Bodenheimer, The Hebrew University, Jerusalem, at their earliest convenvience in order to request the detailed questionnaires of the congress.

The final date for the submission of papers by members is May 15, 1953. The membership fee is IL.10.000. The fee for accompanying wives and children is IL.5.000.

CORRIGENDA (Vol. II)

p. 89: Replace "1. Introduction" by "0. Introduction".

After "for that purpose" at the end of the first paragraph of the Introduction and before "For these reasons . . ." insert:—

1. *Newtonian liquid and Hookean solid.* When the rheological properties of a material are determined by means of the extension of a prismatic specimen, its length l_o gradually increases by $\triangle l$ and this causes its cross-sectional area A gradually to decrease. Therefore, if the load P is kept constant, the tensile traction p

$$p = P/A \qquad (1.1)$$

gradually increases. In tensile tests, the traction is very often referred to the "original" cross-sectional area A_o. For instance in the standard-test of mild steel in the so called "stress-strain diagram", the "stress", sometimes named "nominal stress", is the load divided by the original area

$$p_o = P/A_o \qquad (1.2).$$

Keeping the load P constant and thereby varying the traction during the experiment would seem to complicate the theoretical evaluation. Different devices for keeping the traction constant by gradually reducing the load have therefore been developed by Andrade (1911), Andrade and Chalmers (1932) and Scott Blair and collaborators (1949). However, keeping the load constant has its advantages. Not only is the apparatus considerably simplified, but at the same time there is the advantage that one *single experiment* fulfills the task of a series of experiments with different *p*-s; and diversion from simple Hookean or simple Newtonian behaviour can be detected in *one* experiment.

p. 118, signature should read: F. YARON, S. KERTES and M. HEITNER.

p. 119, l. 2 from bottom: "surface" should read "source".

p. 126, The following should be added: We wish to acknowledge the help extended by Mr. Z. Shiftan of the Geological Institute in the geological interpretations and to Mr. K. Edelstein of the Government Hydrological Service for his help in supplying water samples.

Note to supplement: There is some doubt as to whether the species which has been described as *Alnus orientalis* is correctly classified. The authors are now investigating this classification.

References

Articles

References are to be cited in the text by the author's name and date of publication in parenthesis, e.g., (Taylor, 1932). If the author's name is already mentioned then just the year, appears in the parenthesis, e.g., ... found by Taylor (1932) ... They are to be arranged in alphabetical order and the following form should be used:

3. TAYLOR, G. I., 1932, *Proc. R. Soc.* London, A138, 41.

Book references should be prepared according to the following order:

4. JACKSON, F., 1930, *Thermodynamics*, 4th ed., Wiley, New York.

The title of a paper will appear in the references only if it has been published solely in a local Hebrew journal. This is printed in regular type and is indicated by double inverted commas.

Letters to the Editor

In Letters, references are to be cited in the text by numbers, e.g., Taylor[3] and are to be arranged in the order in which they appear in the text.

TYPOGRAPHY

In all matters of typography the form adopted in this issue should be followed. Particular attention should be given position (of symbols, headings, etc.) and type specification.

ILLUSTRATIONS

Illustrations should be sent in a state suitable for direct photographic reproduction. Line drawings should be drawn in large scale with India ink on white drawing paper, bristol board, tracing paper, blue linen, or blue-lined graph paper. If the lettering cannot be drawn neatly by the author, he should indicate it in pencil for the guidance of the draftsman. Possible photographic reduction should be carefully considered when lettering and in other details.

Half tone photographs should be on glossy contrast paper.

Illustrations should be mounted on separate sheets of paper on which the caption and figure number is typed. Each drawing and photograph should be identified on the back with the author's name and figure number.

The place the figure is to appear should be indicated in the margin of the MS.

PROOFS

Authors making revisions in proofs will be required to bear the costs thereof. Proofs should be returned to the Editor within 24 hours. Otherwise no responsibility is assumed for the corrections of the author.

REPRINTS

Each author will receive 25 reprints free of charge, and additional reprints, may be ordered at the time the first proof is returned. A table designating the cost of reprints is sent with the first proof, but may also be obtained upon request.

Manuscripts should be addressed:
Executive Editor
Bulletin of the Research Council of Israel
P.O.B. 607, Jerusalem